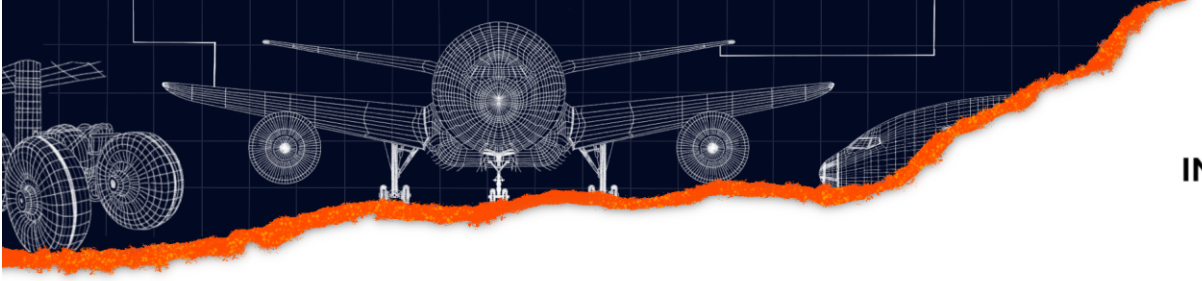

IMPACTO CIENTÍFICO
EDITORA

CLASSICAL MATHEMATICAL MODELS OF POPULATION GROWTH FOR PREDICTING CELL CULTURE IN BIOREACTORS

MODELOS MATEMÁTICOS CLÁSSICOS DE CRESCIMENTO POPULACIONAL PARA PREDIÇÃO DO CULTIVO DE CÉLULAS EM BIORREATORES

MODELOS MATEMÁTICOS CLÁSICOS DE CRECIMIENTO POBLACIONAL PARA PREDECIR EL CULTIVO CELULAR EN BIORREACTORES

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ABSTRACT

Mathematical modeling is an essential tool for predicting results in bioprocesses, especially in cell culture in bioreactors, where there is a high complexity of variables. However, accurately predicting cell behavior at different growth stages remains a challenge. This chapter analyzes four classic deterministic population growth models: Malthus, Verhulst, Montroll, and Gompertz, which can be used to simulate cell growth in bioreactors. The development demonstrates how to solve the ordinary differential equations for each model and adjust the main parameters involved in cell culture. This chapter contributes to a better understanding of classic mathematical models of population growth and also indicates appropriate procedures for modeling in bioprocesses, providing a tool for predicting cell cultures in bioreactors. The study presented paves the way for the development of more comprehensive models to represent different types of cell growth.

Keywords: Deterministic Modeling. Cells. Bioreactors. Population Growth.

RESUMO

A modelagem matemática é uma ferramenta essencial para prever resultados em bioprocessos, especialmente no cultivo celular em biorreatores, onde há uma grande complexidade de variáveis. No entanto, a precisão na predição do comportamento celular em diferentes fases de crescimento ainda é um desafio. Este capítulo tem como objetivo analisar quatro modelos clássicos determinísticos de crescimento populacional: Malthus, Verhulst, Montroll e Gompertz, que podem ser utilizados para simular o crescimento de células em biorreatores. No desenvolvimento foi demonstrado como resolver as equações diferenciais ordinárias de cada modelo e ajustar os principais parâmetros envolvidos no cultivo de células. Este capítulo contribui para o melhor entendimento dos modelos matemáticos clássicos de crescimento populacional e também indica os procedimentos adequados para a modelagem em bioprocessos, oferecendo instrumento para a previsão de

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culturas celulares em biorreatores. O estudo apresentado abre caminho para o desenvolvimento de modelos mais completos para representar o crescimento celular de diferentes tipos.

Palavras-chave: Modelagem Determinística. Células. Biorreatores. Crescimento Populacional.

RESUMEN

El modelado matemático es una herramienta esencial para predecir resultados en bioprocesos, especialmente en cultivos celulares en biorreactores, donde existe una alta complejidad de variables. Sin embargo, predecir con precisión el comportamiento celular en diferentes etapas de crecimiento sigue siendo un desafío. Este capítulo analiza cuatro modelos deterministas clásicos de crecimiento poblacional: Malthus, Verhulst, Montroll y Gompertz, que pueden usarse para simular el crecimiento celular en biorreactores. El desarrollo demuestra cómo resolver las ecuaciones diferenciales ordinarias para cada modelo y ajustar los principales parámetros involucrados en el cultivo celular. Este capítulo contribuye a una mejor comprensión de los modelos matemáticos clásicos de crecimiento poblacional y también indica procedimientos apropiados para el modelado en bioprocesos, proporcionando una herramienta para predecir cultivos celulares en biorreactores. El estudio presentado allana el camino para el desarrollo de modelos más completos para representar diferentes tipos de crecimiento celular.

Palabras clave: Modelado determinista. Células. Biorreactores. Crecimiento Poblacional.

1 INTRODUCTION

Bioreactors are equipment used to transform raw material into product through living organisms such as bacteria, fungi, yeasts, animal cells and their derivatives. Due to the complexity of the bioprocesses, these devices rely on the control of pH, temperature, nutrient concentration, and oxygenation to create an adequate reaction environment (Junior; Cruz, 2012). Thus, the so-called biochemical reactors are extremely useful both for laboratory research and for production on an industrial scale, being present in the development and manufacture of numerous consumer goods, especially in the food and pharmacological areas (Junior; Schmidell; Tonso, 2021).

Bioprocesses involve interactions between biotic and abiotic systems, subject to several physicochemical, genetic and biochemical laws. Thus, mathematical modeling is crucial to understand them, being responsible for representing biochemical reactions through mathematical equations, analyzing the mass balances of each component, and knowing the most economically viable procedures. However, given the complexity of the real processes and the limitations of equation, Bonomi and Morais (2021) suggest that modeling requires constant improvement of techniques and equations to get closer to reality. This characteristic makes it an open field of research with numerous possibilities for development, when applied to bioreactors.

Among the applications of biochemical reactors for the production of pharmacological inputs, the use for the cultivation of animal cells stands out, which can be used as heterologous protein expression systems for therapeutic purposes (Costa, 2013). One of the cells that presents the highest productivity are the cells of the insect *Drosophila melanogaster*. Specifically, the Schneider S2 strain has a lower production cost compared to mammalian cells due to suspension culture, in addition to being able to express mammalian genes due to changes in genetic material. Thus, the protein products of this system have great immunobiological value, allowing the expression of the 80E subunit of the dengue virus and the glycoprotein of the rabies virus (GPV). Thus, this Drosophila strain is the target of studies for the production of new vaccines for the diseases, in addition to being constantly used in the production of receptors, antibodies, enzymes, viral antigens and other products with great relevance for research and production of biological inputs.

According to Barral (2010), the cultivation of S2 cells has as a significant factor of influence the concentration of glucose, since this is the main source of energy in the body. Thus, the inoculum must be prepared in order to meet this need during the process. When the culture is taken to the bioreactor, cell growth can be estimated as a function of glucose concentration, being an alternative to model the bioprocess. There is also evidence that

glutamine is essential for cellular catabolism and anabolism, along with other factors, such as oxygenation, that can be exploited to predict the development of these cells over time.

The cultivation of S2 cells in bioreactors involves the creation of an isolated population of living beings in a controlled environment. Thus, although there are growth limitations imposed by the environment, they are not influenced by other species or external agents. Thus, deterministic models of population growth can be employed to predict crop growth over time and predict parameters that are more appropriate for efficient cultivation.

Among the deterministic models of population growth, Malthus and Verhulst's stand out. The first for being a pioneer and the second for having vast applicability in the growth of other species. In addition to these, Montroll's and Gompertz's models also stand out for contemplating one more variable, the inflection point of the curve, which helps in the simulation of more complex situations than the others. When applying such models, it is necessary to obtain a sample of data that allows the estimation of the parameters in a reliable way, using statistical tools, such as linear regression and the least squares method.

The objective of this chapter is to analyze deterministic models of population growth to predict growth and the factors that interfere in the process of cell production in bioreactors, especially of the insect *Drosophila melanogaster*. To achieve these objectives, the processes of insect cell production in bioreactors were reviewed, the deterministic models of population growth were revisited and the differential equations of population growth for each model were constructed and solved.

2 DETERMINISTIC MODELS OF POPULATION GROWTH

In this section, four well-known classical population growth models are presented and solved, being established by ordinary differential equations (ODE). The resolution processes for each of them were summarized in the following items. The purpose of this study is to present the growth models to be compared with experimental data found in the literature regarding the growth of *Drosophila melanogaster cells*. In this sense, at the end of the chapter, experimental parameters of the growth of this organism were presented.

2.1 MALTHUS'S MODEL

Malthus's model represents the change of a population () with respect to time (t) and is described as a first-order EDO (Equation 1). The variable represents the speed of growth or decrease of the population.$\frac{dP}{dt}u$

$$\frac{dP}{dt} = uP(\mathrm{t}) \qquad (1)$$

P(0) = P0 (2)

Solving the EDO by separable variables, we have:

$\frac{dP}{P} = udt \quad \Rightarrow \quad ln\, ln\, P = ut + c$ (3)

The variable is the constant of the resolution of the indefinite integral and can be obtained through the initial condition given by Equation 2:c

$c = ln\, ln\, P_0 \quad \Rightarrow ln\, ln\, P = ut + ln\, ln\, P_0$ (4)

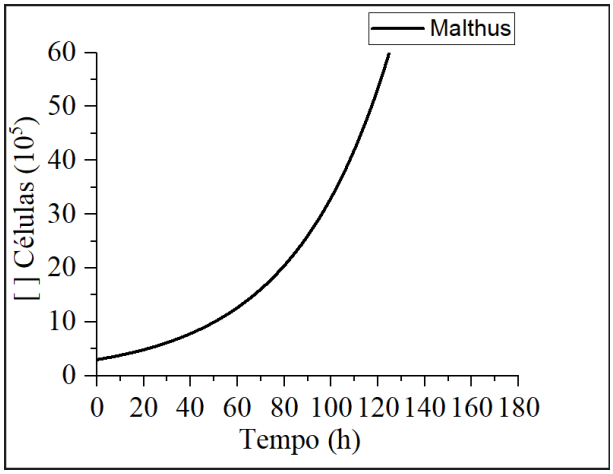
Rearranging Equation 4, we have:

P(t) = P0 e^{ut} (5)

where *P* is the population at any instant *t*, *P is* the initial population, and *u* the speed of growth in between. The typical plot of Malthus's model is shown in Figure 1.

Figure 1

Malthus's model based on equation (5)



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2.2 VERHULST MODEL

The next model studied was developed by mathematician Pierre F. Verhulst, known as the Continuous Logistic Model. According to Bassanezi R. (2010), this model differs from the Malthusian model due to the speed of population growth (). u

In this model, the mathematician considers that the population will grow to a maximum sustainable limit (k), thus, at first an exponential growth is perceived, but from a certain moment the speed of population growth starts to reduce, leading the population to a steady state which will remain unchanged due to environmental inhibitory factors. Thus, the speed taken into account in this model is no longer the direct growth rate, as it varies according to the conditions. The constant now is the intrinsic growth rate of the population, that is, in a situation where there was no inhibition.uu

Thus, the equation of Verhulst's model is obtained from a modification of the Malthusian equation, where the population P is proportional to the population at that instant (Equation 6).

$$\frac{dP}{dt} = \beta(P)P \qquad (6)$$

$$\beta(P) = u\left(\frac{k-P}{k}\right) \qquad (7)$$

Thus, it tends to zero when the population P tends to k, considering that .$\beta(P)u > 0$

Putting Equations 6 and 7 together, we have:

$$\frac{dP}{dt} = u\,P\left(1-\frac{P}{k}\right) \qquad (8)$$

$$P(0) = P0 \qquad (9)$$

Solving Equation 8 by the variable separation method together with the partial fraction integration method, we obtain Equation 10:

$$ln\ ln\,|P| - ln\ ln\,|k-P| = ut + c \qquad (10)$$

Applying the initial condition of Equation 9, we have:

$$c = ln\ ln\left|\frac{P_0}{k-P_0}\right| \qquad (11)$$

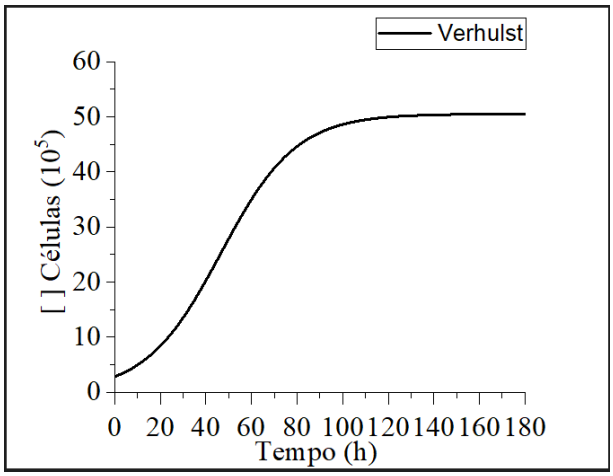
Substituting Equation 11 into Equation 10 and isolating P(t) gives Equation 12:

$$P(t) = \frac{k\,P_0}{(k-P_0)e^{-ut}+P_0} \qquad (12)$$

The graph of the Verhulst model is shown in Figure 2.

Figure 2

Verhulst's model based on equation (12)



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2.3 MONTROLL MODEL

In 1971, according to Dentamaro A. (2019), the American scientist and mathematician Elliott Waters Montroll published a new model for studying the population known today as the Montroll Model. This is similar to Verhulst's model in relation to the variability of the population growth rate, now called , and the existence of a sustainable upper limit (K), however, this relationship is no longer linear, it is related to a parameter λ, which indicates the inflection point of the studied curve. r

Thus, Montroll's Model is given by:

$$\frac{dP}{dt} = rP\left[1 - \left(\frac{P}{K}\right)^{\lambda}\right] \qquad (13)$$

$$P(0) = P0 \qquad (14)$$

Where, > 0, λ > 0, and when λ=1 the equation corresponds to Verhulst's model.r

To solve this ODE, at first, the equation was rearranged as follows:

$$\frac{dP}{dt} - rP = -\frac{r}{k^{\lambda}}P^{\lambda+1} \qquad (15)$$

Subsequently, Bernoulli's Linearization method was applied, obtaining:

$$\frac{dP}{dt}P^{-\lambda-1} - rP^{-\lambda} = -\frac{r}{k^{\lambda}} \qquad (16)$$

Then, the replacement was made by Equation 17 and the terms were reorganized, arriving at Equation 18:

$$u = P^{-\lambda} \qquad (17)$$

$$u' + \lambda r u = \frac{\lambda r}{K^{\lambda}} \qquad (18)$$

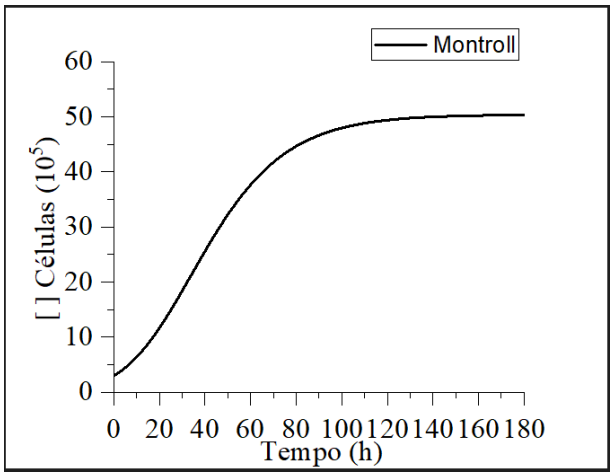
Solving Equation 18 by the Integrating Factor method, returning the variable P and using the initial condition given by Equation 14 to obtain the integration constant, it can be seen that the solution of Equation 13 is:

$$P(t) = \frac{K P_0}{[P_0^{\lambda} + (K^{\lambda} - P_0^{\lambda})e^{-\lambda r t}]^{\frac{1}{\lambda}}} \qquad (19)$$

The Montroll model graph is shown in Figure 3.

Figure 3

Montroll model based on equation (19)



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2.4 GOMPERTZ MODEL

Another model widely used for the simulation of cell growth is the one proposed by the English mathematician Benjamin Gompertz, which, unlike the previous ones, considers the growth velocity (as a logarithmic equation. Thus, the EDO that represents it, according to Dentamaro A. (2019), is:r)

$\frac{dP}{dt} = rP - \lambda P ln|P| = P(r - |P|)$ (Equation 20)

P(0) = P0 (Equation 21)

In addition to relating the speed of growth, Gompertz's model also uses as a parameter the inflection point of the curve (and the maximum sustainable limit of the population (K), which occurs when the rate of population variation is zero:λ)

$\frac{dP}{dt} = P(r - |P|) = 0$ (22)

$ln|P| = \frac{r}{\lambda}$ (23)

$K = e^{\frac{r}{\lambda}}$ (24)

To solve Equation 20, a substitution was initially performed for , as follows: $Q = ln\ ln\ |P|$

$\frac{dQ}{dt} = \frac{1}{P}\frac{dP}{dt}$ (25)

$\frac{dQ}{dt} = r - \lambda Q$ (26)

Thus, from the method of separable variables and subsequent return to the variable P, it was possible to determine the general solution of the ODS:

$-\frac{1}{\lambda} ln\ |r - \lambda\ ln\ ln\ |P||\ = t + c$ (27)

Using the initial condition of Equation 21 it can be concluded that the constant is:c

$c = -\frac{1}{\lambda} ln\ |r - \lambda\ ln\ ln\ |P_0||$ (28)

Therefore, the solution of equation 20 is:

$-\frac{1}{\lambda} ln\ |r - \lambda\ ln\ ln\ |P||\ = t - \frac{1}{\lambda} ln\ |r - \lambda\ ln\ ln\ |P_0||$ (29)

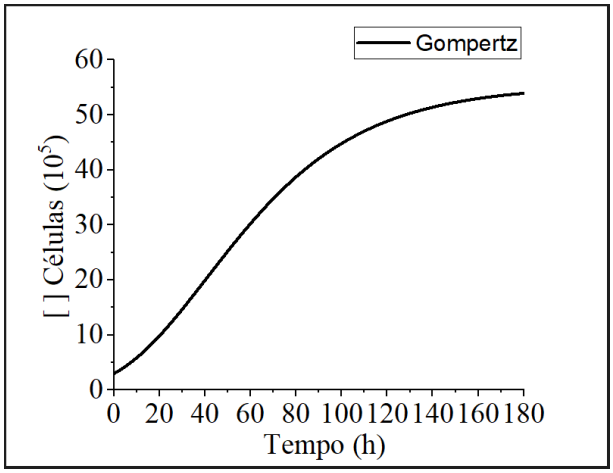
$P(t) = e^{\frac{r}{\lambda}}\ exp\ exp\left[-e^{-\lambda t}\left(\frac{r}{\lambda} - ln\ ln\ |P_0|\right)\right]$ (30)

$P(t) = K\left(\frac{P_0}{K}\right)^{e^{-\lambda t}}$ (31)

The graph of the Gompertz model is shown in Figure 4.

Figure 4

Gompertz model based on equation (31)



Source: authors.

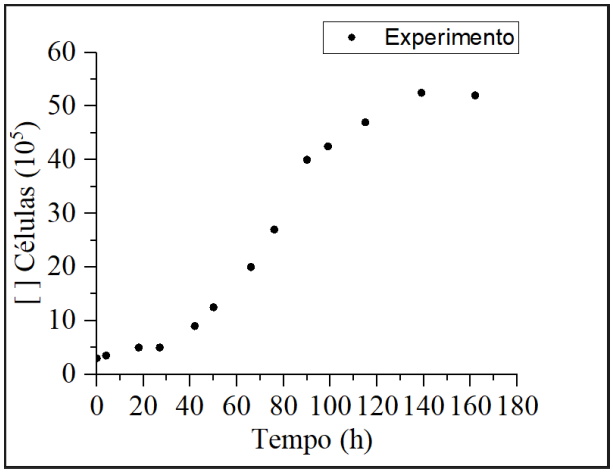
2.5 DATA FOR THE SIMULATIONS

In addition to population growth equations, it is common to use experimental databases. As a suggestion for the application of the models, Galesi A. (2007) presented the growth behavior of Drosophila Melanogaster cells of Schneider S2 lineage under different conditions. The most satisfactory experiment used TC100 medium supplemented with 3 g/L of yeastolate, 10 g/L of glucose, 1% (v/v) of lipid emulsion, 3.5 g/L of glutamine, 0.1 % (w/v) of Pluronic F68 and 30% of dissolved oxygen. In addition, the bioreactor agitation was set to 50 rpm up to 6.5 hours, 75 rpm up to 69.7 hours, and 90 rpm for the remainder of the experiment. The bioprocess started with a population of 3,105 cells per mL and had an average growth rate of 0.024 $^{h-1}$ In addition, a significant synthesis of rabies virus glycoprotein (GPV) can be noted.

The cell growth data described by Galesi A. (2007) are presented in the curve in Figure 5, built based on the author's data using the Origin 2024b software.

Figure 5

Cell Growth Curve. Experimental data



Source: prepared by the authors based on Galesi A. (2007).

3 CONCLUSIONS

This study focused on the construction of deterministic models of population growth for the evaluation of cell culture, especially of the S2 cell of Drosophila melanogaster in bioreactors. The general objective, to analyze these models to predict growth and the factors that interfere with the production of S2 cells, was fully achieved. In addition, the specific objectives of reviewing the production processes, the models and building and solving the differential equations with parameter adequacy were fully met throughout the research.

The analysis of Malthus' model demonstrated that it is effective in simulating cell growth only in the initial exponential phase of culture. This limitation is due to the fact that the Malthusian model does not consider nutrient constraints and accumulation of metabolic products, which inevitably lead to slowing down and subsequently to the stationary phase in a bioreactor environment.

Verhulst's model presents a curve that resembles the experimental one. Although Verhulst's model incorporates the carrying capacity of the environment, its stationary phase tends to infinity, which diverges from biological reality, where cell mortality exceeds birth rates in the long term, leading to a decline in the population.

Montroll's model is able to represent the exponential, transitional, and stationary phases of cell growth. However, the exponential growth speed is considerably higher than the experimental one. Notably, this model also fails to simulate the decline phase, a crucial aspect in real population dynamics.

Finally, Gompertz's model demonstrates high efficacy in predicting cell behavior during the stationary phase, aligning well with the trend observed experimentally. However, its main limitation was the inability to capture the initial sharp exponential phase, resulting in a less variable growth speed and far from reality in the first hours of cultivation, when nutrient availability is high.

In summary, the study reported in this chapter reaffirms the validity and importance of classical mathematical models as indispensable tools for understanding population growth in bioprocesses. Despite the limitations inherent to each model in capturing the full complexity of cell dynamics in all its phases, its application allows a fundamental prediction of culture behavior and the identification of crucial parameters for process optimization.

The research contributes significantly to the field of biotechnology by providing a mathematical procedure for predicting cell growth in bioreactors. This can help in the development of more efficient strategies for cultivation, in the optimization of operating conditions, and in the scaling of the production of inputs of high biological value. In a future scientific paper, the authors intend to compare the developed models with a set of experimental data.

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