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MODELING THE GROWTH CURVE OF PHOTOFERMENTATIVE HYDROGEN-PRODUCING BACTERIA

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ABSTRACT

Sustainable energy production is a current debate. Using microorganisms to produce hydrogen, a renewable energy source, has been the focus of research efforts. Among several parameters influencing biohydrogen production via photofermentation, intrinsic characteristics of the microorganism, such as inoculum age and growth phase kinetic are among the determining factors. Therefore, the study of growth curves of bacteria *Rhodobacter capsulatus* and *Rhodospirillum rubrum*, two species known to be applicable in photofermentation, and their main kinetic parameters were analyzed using the modified Gompertz model. The growth curves for *Rhodobacter capsulatus* and *Rhodospirillum rubrum* were determined and fitted with the predictive model. The duration of the *lag* phase (λ), generation time (Tg), maximum specific growth rate (μ_m), and maximum biomass concentration were 107.5 h, 50.7 h, 0.014 h^{-1}, and 1.05 g/L for *R. capsulatus* and 8.36 h, 95.22 h, 0.007 h^{-1}, and 1.22 g/L for *R. rubrum*.

Keywords: Growth kinetics. Modified Gompertz model. Biohydrogen. Photofermentation.

1 INTRODUCTION

The climate emergency is a recent concern of governments and society. The increase in global temperature caused by the emission of polluting gases from the burning of fossil fuels intensifies the discussion of replacing energy matrices with renewable sources [1]. In this context, hydrogen emerges as a sustainable alternative to fossil fuels, as it is renewable and does not produce polluting gases during combustion. However, the methods employed for its production are not always free from CO2 emissions[2]. Thus, hydrogen production through biological routes has been pointed out as a potential solution for clean gas production[3]. Among the biological routes explored, photofermentation with non-sulfur purple bacteria (PNS) has gained prominence in discussions. Although highly promising, photofermentative hydrogen production still needs to overcome some challenges to become commercially competitive, including low production rates and costs related to the increase in the photobioreactor[4]. In this sense, the rate and yield of hydrogen production depend heavily on the carbon source used and physiological growth conditions, such as light sources and distribution, and bacterial growth mode[5].

The study of microbial growth kinetics is essential for the development of any bioprocess. Therefore, predictive microbiology is widely employed to describe the growth curve of microorganisms and their associated parameters. For PNS bacteria, models such as the logistic have been used to describe the growth of *Rhodobacter sphaeroides*[6] and *Rhodobacter capsulatus*[7] and the modified Gompertz in the kinetics of *Rhodobacter capsulatus*[8]. The application of these growth models of PNS bacteria allows for a more accurate prediction of the stage at which the microorganism is and its main kinetic parameters related to growth, thus contributing to the development of a viable large-scale biohydrogen production process by photofermentation.The current study aimed to evaluate the fitting of the modified nonlinear Gompertz model and its kinetic parameters to the growth of PNS cultures, *Rhodobacter capsulatus* and *Rhodospirillum rubrum*.

2 MATERIAL & METHODS

The strains used were *Rhodobacter capsulatus* DSM 1710 and *Rhodospirillum rubrum* DSM 467, obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ).

The microorganisms were grown in RCV basal medium, containing the following composition per liter: 4.02 g of $C_4H_6O_5$, 0.60 g of KH_2PO_4, 0.90 g of K_2HPO_4, 0.12 g of $MgSO_4.7H_2O$, 0.075 g of $CaCl_2.2H_2O$, 0.02 g of $Na_2EDTA.2H_2O$, 0.001 g of thiamine, and 1 mL of micronutrients[10]. After dissolving the reagents, the pH of the medium was adjusted to 6.8 ± 0.2 and then sterilized at 121°C and 1 atm for 20 minutes. Subsequently, the bacteria were inoculated into 1000 mL Schott bottles, and purged with argon gas to maintain the cells under anaerobic conditions. The culture bottles were incubated with an initial cell density of 0.2 g/L in an incubator set at 30°C with a light intensity of 3,500 lx provided by fluorescent lamps.

The monitoring of cell growth was performed by periodically collecting the reaction medium. Samples of 10 mL were collected and centrifuged for 12 min at 7730 g. Subsequently, the sediment (biomass) was used for optical density readings at a wavelength of 660 nm. The readings were performed in duplicate and converted into cell concentration (g/L) using previously established calibration curves. These curves correlate absorbance readings with the dry mass of cells, the latter being based on the gravimetric method of analysis[9].

The bacterial growth curves were fitted to the modified Gompertz mathematical model, as demonstrated in Equation 1[10]. The software used for the modeling application was Statistica 8.0 (Statsoft Inc., Tulsa, OK, USA). The parameters obtained from the modeling (a, b, and c) were used to determine the kinetic parameters of *lag* phase duration (λ), maximum specific growth rate (μ_m), and generation time (Tg), as per Equations 2, 3, and 4, respectively.

$$y = a.exp\,[-exp(b - c.t)] \quad (1)$$

$$\lambda = \frac{(b-1)}{c} \quad (2)$$

$$\mu_{max} = \frac{a.c}{e} \quad (3)$$

$$Tg = \frac{ln\,(2)}{\mu_m} \quad (4)$$

Where: $y = \ln\,(N/N_0)$, with N being the cell concentration at time t and N_0 being the initial cell concentration; and a, b e c = model parameters, where $a = \ln\,(N_\infty/N_0)$, model's asymptote.

3 RESULTS & DISCUSSION

The Gompertz model was used to evaluate the growth of the selected strains in this study when exposed to fluorescent lighting at 30°C for 264 hours.

According to the data presented in Figure 1, a sigmoidal growth of *R. capsulatus* bacteria is observed, with experimental data fitting well to the Gompertz model (R^2 = 0.9916). The parameters obtained from the model indicate a *lag* phase duration of 107.5 hours, a specific growth rate of 0.014 h^{-1}, and a generation time of 50.7 hours. Additionally, according to the asymptotic value (a= 1.65456), the maximum biomass concentration was 1.05 g/L.

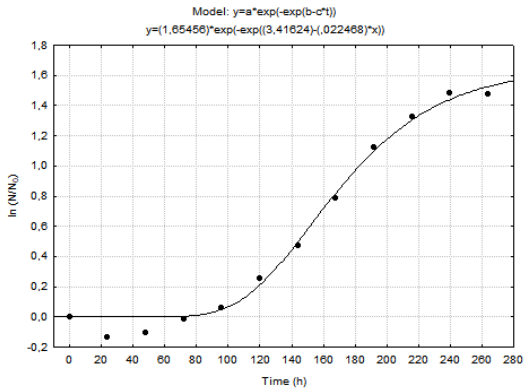

Figure 1 Sigmoidal curve fitting of the Gompertz model to the data obtained from the growth of *R. capsulatus*.

In Figure 2 it is possible to observe a smaller adjustment of the growth of the bacteria *R. rubrum* to the Gompertz model, with the coefficient of determination being 0.97. According to the predictive model, the *R. rubrum* strain exhibited a *lag* phase of 8.36 hours, indicating a rapid adaptation to the medium compared to *R. capsulatus*. This stage is unpredictable, with its duration influenced by factors such as bacterial phenotype, inoculum size, physiological state of the population, and physicochemical changes in the environment [5]. Furthermore, according to the model's asymptote (a = 1.805361), the cell concentration reached a maximum value of 1.22 g/L. However, during the exponential phase, the specific growth rate was 50% of that obtained by *R. capsulatus*, that is 0.007 h^{-1} and the generation time was 95.22 hours.

Several mathematical models are proposed to fit microbial growth curves. Among them, two popular mathematical models reported in the literature are the Gompertz model and the Logistic model. The modified Gompertz model was used to fit experimental growth data of two strains of *R. capsulatus* in a bioreactor with a modified RCV medium illuminated by a sodium vapor lamp and at a temperature of 30°C [8]. Under these conditions, the coefficient of determination (R^2) was 0.99, resulting in μ_{max} values of 0.195 h^{-1} and 0.166 h^{-1}, generation times (Tg) of 3.55 hours and 4.18 hours, and *lag* phase durations (λ) of 9.8 hours and 13.9 hours. The logistic model was used to describe the growth of *R. capsulatus* bacteria under different light intensities and temperatures, resulting in a maximum biomass concentration of 0.70 g/L and an apparent specific growth rate (k_C) of 0.059 h^{-1} under conditions of light intensity of 3000 lx and 30°C[7].

Understanding the growth kinetics of these microorganisms is crucial for optimizing biohydrogen production, as the highest yield of this gas is associated with the late exponential and early stationary growth phases in photosynthetic non-sulfur bacteria (PNS)[11].

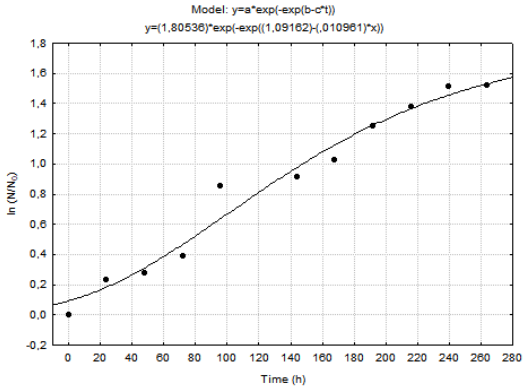

Figure 2 Sigmoidal curve fitting of the Gompertz model to the data obtained from the growth of *R. rubrum*.

4 CONCLUSION

Based on the obtained results, it is concluded that the predictive modeling proposed by the modified Gompertz model significantly fitted (R^2 = 0.9916 for *R. capsulatus* and R^2 = 0.96 for *R. rubrum*) the experimental growth data of hydrogen-producing cultures when exposed to fluorescent light and at a temperature of 30°C. Understanding the kinetics of a bioprocess is of vital importance to optimize its variables and achieve higher yields. In this study, growth curves and kinetic parameters of two important PNS strains were demonstrated. It is expected that these data will contribute to understanding the growth behavior of these strains and serve as a basis for establishing a productive and economically viable bioprocess.

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