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Mathematical Modeling of Citric Acid Production and Microbial Growth Kinetics Using *Aspergillus niger* in Batch Fermentation

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Abstract. This study investigates the microbial growth kinetics and citric acid production by *Aspergillus niger* in batch fermentation, employing Monod, Logistic, and Gompertz models to analyze the dynamic relationships between biomass growth, substrate consumption, and citric acid formation. A series of experiments were conducted in a 5 L stirred tank batch bioreactor with 3 L working volume, using glucose (97 g/L) as the carbon source. Model predictions were validated using experimental data from batch fermentation runs. The results showed that *A. niger* exhibited typical microbial growth behavior, with biomass concentration increasing from 2.65 g cell/L at 0 hours to a peak of 14.58 g cell/L at 192 hours, before declining to 12.39 g cell/L by the end of the fermentation. Citric acid production followed a similar trend, reaching a maximum of 24.75 g/L at 240 hours and subsequently declining. Model fitting results revealed that the Gompertz model provided the best fit and yield, maximum specific growth rate μ_{max} 15 h^{-1} exhibiting the highest goodness-of-fit ($R^2 = 0.989$) compared to the Monod ($R^2 = 0.974$) and Logistic models ($R^2 = 0.988$). The Gompertz model effectively captured the S-shaped growth and product formation patterns, including the deceleration phase associated with nutrient depletion and product inhibition. This study highlights the effectiveness of the Gompertz model in describing the fermentation process and emphasizes the importance of model selection in evaluating fermentation performance and improving the understanding of citric acid production kinetics.

1 Introduction

Citric acid, also known as 2-hydroxy-1, 2, 3-propane-tricarboxylic acid, derives its name from the Latin word citrus, referring to plants within the Citrus genus, including lemon trees [1]. It is a colorless, weak organic acid that is highly soluble in water in its pure form. This acid can be produced from both natural and synthetic sources, and its global production has significantly increased in recent years. In 2020, global citric acid production reached 2.39 million tons, with projections indicating an increase to 2.91 million tons by 2026 [2]. This weak acid is widely used in various industries, such as food and beverage, medicines, cosmetics, and animal feed, due to its versatile nature. In the food industry, citric acid is commonly used as an acidulant due to its high solubility and low toxicity. It is also utilized for cleaning specialized steam boilers. Within the beverage, food, and confectionery industries, citric acid serves as an antioxidant, emulsifier, buffer, chelating agent, flavor enhancer, and acidulant [3].

Citric acid can be extracted from natural sources such as lemons, guavas, and oranges, or produced through microbial fermentation. However, due to the insufficient yields from plant sources to meet global demand, microbial fermentation has become the primary method for citric acid production. A wide variety of microorganisms, including bacterial and fungal species, are employed in large-scale citric acid production. These include *Bacillus licheniformis*, *Arthrobacter paraffinens*, *Penicillium janthinellum*, *Candida species*, *Yarrowia lipolytica*, *Hansenula anamola*, and various strains of the *Aspergillus* genus [4]. Among these, *Aspergillus niger* has become the preferred microorganism for large-scale citric acid production due to its ability to utilize a diverse range of substrates and its high citric acid production capacity [5]. Research has shown that *A. niger* can produce up to 90 g/L of citric acid through submerged fermentation when using sucrose as the carbon source [6].

Citric acid is particularly important in the food sector, where it accounts for a significant portion of global consumption. In 2016, it comprised 62.53% of global citric acid consumption within the food and beverage industry, shown in Figure 1., with an anticipated growth rate of 6.03% from 2016 to 2021 [5]. In the pharmaceutical industry, this acid is essential in regulating mineral formation and stimulating nerve cells [9]. It is also used in the treatment of kidney stones [10] and is a key ingredient in drug formulations and healthcare products [11]. In the cosmetics industry, this compound functions as an antioxidant and exfoliant, improving product stability and absorption [12].

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Additionally, in agriculture, it enhances nutrient absorption and stimulates plant growth. Furthermore, citric acid is utilized in the textile and detergent industries for cleaning and descaling equipment. A 2022 evaluation of the global citric acid market projected that its market value would grow at an annual rate of 3.82% through 2032 [13].

Despite its widespread application, the kinetic understanding of citric acid production remains a significant challenge due to the complexities of the fermentation process. This complexity arises from the wide variety of substrates that can be utilized, the dynamic interactions between *A. niger* and these substrates, and the inhibitory effects exerted by both substrates and products. Such factors complicate the development of a universal kinetic model capable of accurately describing citric acid fermentation. Nevertheless, mathematical modeling has proven valuable for predicting system behavior, identifying optimal bioprocess designs, and defining key operating parameters, ultimately enhancing process efficiency [14]. To support this, Woinaroschy et al. studied and improved kinetic models for citric acid fermentation using molasses and whey, comparing different models with experimental results. Their findings underscored the critical influence of substrate concentration and inhibition effects on fermentation performance[15, 16]. Building upon this foundation, Sharifzadeh Baei et al. applied the logistic model to describe microbial growth and incorporated the Luedeking-Piret equation to model citric acid production from apple pomace by *A. niger*, revealing that citric acid synthesis in this system is closely associated with microbial growth [17]. Complementing these efforts, Baei et al. and other researchers have reported essential kinetic parameters, including the maximum specific growth rate (μ_{max}), yield coefficients, and substrate affinity constants across different microbial strains and fermentation conditions. These insights have provided a more detailed understanding for the calibration and refinement of fermentation models [17, 18]. By utilizing these mathematical modeling methods, researchers can significantly reduce the need for extensive empirical experimentation under varying conditions, resulting in notable savings in both time and research costs while accelerating process development.

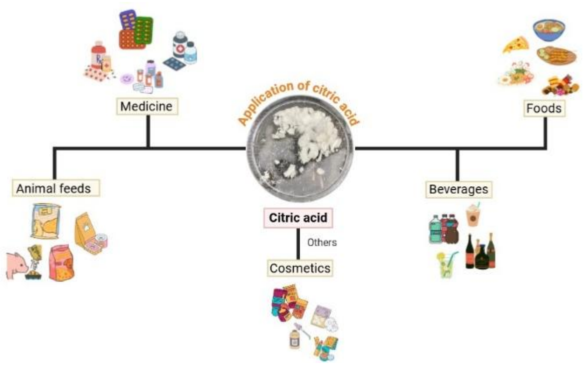


Fig. 1. Applications of citric acid in various industries

In recent years, scientific investigations into *A. niger* fermentation have focused on enhancing citric acid production due to its substantial industrial importance and the organism's favorable metabolic characteristics, such as high citrate synthase activity and the ability to metabolize a wide spectrum of carbon sources [13]. These studies have systematically examined both fermentation parameters and genetic improvements to elevate citric acid yields [13]. To quantitatively characterize and evaluate *A. niger* growth and metabolite formation, several mathematical models have been applied.

The Monod model is widely used to describe microbial growth as a function of substrate concentration and has been applied to *A. niger* fermentations for estimating essential kinetic parameters such as maximum specific growth rate and substrate affinity [18, 19]. In addition, the Logistic model has been effectively utilized to capture the typical sigmoidal growth patterns observed in *A. niger*, particularly under nutrient-limited conditions. This model has proven useful for fitting empirical data and estimating maximum biomass concentrations in both submerged and solid-state systems [20]. The Gompertz model, which has gained increasing attention in the field of fungal kinetics, offers a comprehensive description of the lag, exponential, and deceleration phases of growth. It has demonstrated a strong ability to fit data related to biomass accumulation and citric acid production, especially under conditions exhibiting S-shaped growth curves [21]. Comparative assessments indicate that while each model possesses specific advantages depending on the fermentation context, the Gompertz and Logistic models are particularly well-suited to capture the complex kinetics of filamentous fungi like *A. niger* [20, 21]. Furthermore, some studies have introduced alternative or hybrid modeling approaches to improve predictive accuracy in specialized fermentation scenarios [13]. Overall, the use of these mathematical models plays a pivotal role in refining process control, improving yield prediction, and reducing experimental effort in industrial-scale citric acid fermentation processes [13, 19, 20].

Within this context, the present study proposes and evaluates the Monod, Logistic, and Gompertz kinetic models to describe citric acid production and biomass formation by *A. niger* in batch fermentation systems. The investigation involves monitoring changes in biomass, glucose concentration, and citric acid levels to assess the models' ability to capture experimental trends. These models aim not only to describe microbial behavior but also to facilitate improved control strategies and efficiency in scale up fermentation operations.

2 Material and methods

2.1 Chemicals

The Potato Dextrose Broth (PDB) and Potato Dextrose Agar (PDA) were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. D-glucose ($C_6H_{12}O_6$), monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), calcium chloride ($CaCl_2$), and calcium carbonate ($CaCO_3$) were purchased from Sisco Research

Laboratories Pvt. Ltd., Taloja, India. Urea was obtained from Honeywell International Inc., New Jersey, USA. Tween 80 was purchased from Scharlab S.L., Sentmenat, Spain. 3, 5-Dinitrosalicylic acid was procured from Sisco Research Laboratories Pvt. Ltd., Taloja, India. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from QReC™, USA.

2.2 Microorganism and inoculum

A. niger TISTR 2365 were obtained from Thailand Institute of Scientific and Technological Research (Pathumthani, Thailand). Fungal strain was inoculated into Erlenmeyer flask containing 100 mL Potato Dextrose Broth (PDB) and maintained at 4°C for stock cultures.

2.2.1 Inoculum preparation

A. niger TISTR 2365 from stock cultures were transferred to sterile plate containing Potato dextrose agar (PDA). The cultures were incubated at 30°C in control incubater (Shaker Incubator, INC 125 FS digital, IKA-Werke GmbH & Co. KG., Staufen, Germany). After 168 hours the cultures were used as inoculum for batch cultivations in shake flask.

2.2.2 Starter preparation of A. niger

The PDA culture of *A. niger* was prepared by directly suspending the spores from the mycelium growth in the modified media. The spore suspension of *A. niger* was prepared in sterile Tween 80 to a final concentration of 3 x 10^8 spores/mL using a Thomas hemocytometer.

2.3 Kinetics of growth and citric acid production

2.3.1 Media Composition

The medium consisted of glucose ($C_6H_{12}O_6$) at concentrations of 97 g/L of D-glucose, 1 g of urea, 0.3 g/L of KH_2PO_4, 0.3 g/L of K_2HPO_4, 0.3 g/L of $MgSO_4 \cdot 7H_2O$, 0.1 g/L of $CaCl_2$. The pH of the medium was adjusted to 4 using hydrochloric acid. The medium was prepared and transferred into a batch bioreactor with a working volume of 3 L. The medium was sterilized by autoclaving at 121 °C for 15 minutes at 15 lb/in^2 to ensure aseptic conditions before inoculation.

2.3.2 Citric Acid Production and Kinetic Analysis of A. niger TISTR 2365 in the Stirred Tank Bioreactor

The bioreactor system was assembled in accordance with the manufacturer's guidelines and connected to all necessary operational equipment. The fungal inoculum was prepared following the procedure outlined in Section 2.2.1, using the optimized culture conditions. To ensure reproducibility and reliability of the results, the fermentation process was carried out in triplicate. A volume of 300 mL of fungal pre-culture was inoculated into the 5 L batch bioreactor containing 3 L of sterilized liquid medium in the bioreactor (MDFT-N-5L, B.E. MARUBISHI CO., LTD., Bangkok Thailand) accordingly to the setup. The cultivation was initiated under the specified conditions: agitation speed of 300 rpm with 1L/min aeration rate at 30°C, and sampling was performed every 48 hours. Each sample, with a volume of 50 mL, was collected over a total cultivation period of 384 hours to monitor fungal growth kinetics and citric acid production.

Fig. 2. Citric acid production in 5 L bioreactor using *A.niger* grown in modified media.

2.4 Analytical measurement

2.4.1 Analytical Techniques

Fermentations were performed in triplicate. Samples were withdrawn at the end of the fermentation period. The broth culture was centrifuged at 15,000 rpm to separate the mycelia. The collected filtrate was used to estimate residual sugar and citric acid production. The dry weight of mycelium was obtained by filtering broth samples through pre-weighed filter discs. Biomass concentration was determined by dry weight measurement. The harvested biomass was washed with 0.85% saline solution, followed by deionized water, then dried overnight at 80°C, cooled in a desiccator, and weighed [22]. Sugar concentration was measured using the dinitrosalicylic acid (DNS) method [23], while citric acid was quantified by titration with 0.1 N sodium hydroxide using phenolphthalein as an indicator [24]. The volume change of the sample solution from colorless to pink to red was recorded, calculated, and reported as the citric acid concentration.

2.4.2 Investigation of Growth Kinetics of A. niger

The study focused on analyzing the growth kinetics of *A. niger* during fermentation, with particular attention to biomass production, citric acid formation, and glucose consumption. All measurements in this study were conducted in triplicate to ensure accuracy and reproducibility of the results. Key experimental rates were calculated based on data collected over a 384-hour fermentation period. These include rate of biomass production r_x, where (r_x = d[X]/dt) and [X] represents biomass concentration, the rate of citric acid formation r_p, where (r_p = d[P]/dt) and [P] is citric acid concentration, and glucose consumption rate r_s, where (r_s = d[S]/dt) and [S] denotes glucose concentration.

2.5 Comparison of model kinetics

Predicted data with the consideration of the mathematical modeling using OriginPro V.2021 (OriginLab corporation, MA, USA) for evaluating parameter values in the context of nonlinear regression.

3 Mathematical modelling

Mathematical models are essential for explaining the citric acid production process through fermentation by the microorganism *A. niger*. The application of these mathematical models aids in the calculation and analysis of cell growth, sugar consumption, and product formation. This approach enables researchers to determine effective kinetic parameters that enhance fermentation yield. In this study, three mathematical models, namely the Monod Model, Logistic Model, and Gompertz Model, were applied to fit the experimental data of the fermentation process. Kinetic parameters were determined through the examination of experimental profiles and their corresponding production. The validity of the models was confirmed using three independent sets of experimental data, encompassing biomass production and product formation. The equations for biomass production models are defined in Eq. 1-3.

Monod kinetics is a model that describes the growth of microorganisms based on the concentration of the substrate available in the system. It employs an equation that expresses the growth rate as a function of the substrate concentration and a saturation value, which represents the maximum growth rate. When the substrate concentration reaches a certain level, the microorganisms reach their maximum growth capacity, and the growth rate no longer increases. The equations for monod kinetics are given in Equation 1:

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \tag{1}$$

where: μ is the specific growth rate (h^{-1})
μ_{max} is the maximum growth rate (h^{-1})
S is the substrate concentration (g/L)
K_S is the Monod constant, which indicates the substrate concentration at which the microorganism grows at half of its maximum rate.

The Logistic Model is another mathematical model used to describe S-curve growth, which is similar to the Gompertz model but simpler in terms of calculations and explaining the growth with a limiting factor. In this model, the population or product grows rapidly in the initial stages and then gradually slows down as it approaches the maximum growth limit, also known as the carrying capacity. The equations for logistic kinetics are shown in Equation 2:

$$\ln\left(\frac{X}{X_0}\right) = \frac{a}{1 - exp(b - cx)} \tag{2}$$

where: X is the population size or cell concentration at time (g/L)
X_0 is the initial population size (initial cell concentration) (g/L)
a is a constant that reflects the difference between the maximum population size and the initial population size.
b is a constant that relates to the initial growth rate (h^{-1})
c is a constant that defines the rate of growth slowdown (h^{-1})
t is the time (h)

The Gompertz Model is a widely used mathematical model to describe growth processes exhibiting an S-curve. It begins with slow growth, accelerates, and then decelerates as the system approaches its maximum limit. In biological production processes or the growth of living organisms (such as microorganisms) or population dynamics, growth is often limited by resource constraints, preventing continuous expansion without bounds. The Gompertz equation is expressed are:

$$\ln\left(\frac{X}{X_0}\right) = a\ exp\{-exp(b - ct)\} \tag{3}$$

where: X represents the population size or cell concentration at time (g/L)
X_0 is the initial population size (initial cell concentration) (g/L)
a is a constant associated with the maximum growth rate
b is a constant that defines the starting point of growth (h^{-1})
c is a constant related to the rate of growth slowdown (h^{-1})
t is the time (h)
To assess the biological behavior of the fermentation process, the modified Gompertz model was employed to estimate key kinetic parameters related to citric acid and biomass formation. This model was selected based on its superior statistical performance compared to alternative models. The primary biological parameters evaluated include the maximum production potential (A), the specific formation rate (μ), and the duration of the lag phase (λ). These were calculated using multi-parametric nonlinear regression based on the modified Gompertz equation as proposed by Zwietering et al. (1990), which is expressed as follows Equation 4:

$$y = A \exp\left\{-\exp\left[\frac{\mu e}{A}(\lambda - t) + 1\right]\right\} \tag{4}$$

In this equation: y represents the citric acid or biomass concentration (g/L) at a given time t (days)
A (g/L) denotes the asymptotic maximum concentration
μ (g/L/day) is the specific citric acid (or biomass) formation rate
λ (days) represents the lag phase duration
The use of mathematical models helps in analyzing the microbial growth behavior and product formation in batch fermentation processes. The parameters obtained from these models can help predict and control the fermentation process, thus improving the efficiency of biomass production and metabolite formation.

3.1 Complete Model for Parameter Estimation

In this study, a system of three Ordinary Differential Equations (ODEs) is used to represent the changes in biomass concentration [X], substrate concentration [S], and product concentration [P] based on batch experimental data. These equations describe the dynamic relationships of these components during the fermentation process. The system of equations is as follows:

- The rate of change of biomass concentration:

$$\frac{dX}{dt} = \mu \cdot X - k_d \cdot X$$

- The rate of change of substrate concentration:

$$\frac{dS}{dt} = \frac{1}{Y_{X/S}} \cdot \mu \cdot X - m_s \cdot X$$

- The rate of change of citric acid concentration:

$$\frac{dP}{dt} = Y_{P/X} \cdot \mu \cdot X - \mu_P \cdot X$$

In these equations, the following parameters are used:

- $Y_{X/S}$: The yield coefficient of biomass per substrate (g/g)
- $Y_{P/X}$: The yield coefficient of product (citric acid) per biomass (g/g)
- m_S: The maintenance coefficient for substrate utilization (-)
- k_d: The death rate coefficient of microorganisms (h^{-1})

These equations and parameters are crucial for modeling the fermentation process and estimating key parameters that determine the efficiency of citric acid production under the given experimental conditions.

4 Result and discussion

4.1 Model validation and parameter estimation

The batch fermentation model was validated using experimental data obtained throughout the course of this study. This validation was accomplished by estimating the parameters of the various kinetic models under experimented runs over fermentation time. Table 1. displays the estimated parameters along with the optimal values for the kinetic models considered. The estimation of the maximum specific growth rate (μ_{max}) using both the Logistic and Gompertz models yielded similar results. However, the Monod model provided the highest μ_{max} value of 19.08 h^{-1}. For the substrate affinity constant (K_s), the Monod model yielded a result of 93.98 g/L. Figure 3. showed the mathematical model graphs for three models: Monod, Logistic, and Gompertz, using data for substrate concentration (sugar), biomass (dry *A. niger* cells), and product (citric acid).

The main objective of the fermentation process is to maximize substrate utilization, thereby achieving the highest possible yield. To ensure that fermentation proceeds under optimal conditions, it is crucial to develop an accurate mathematical model and precisely determine the associated parameters. This model should include equations that describe cell growth, substrate consumption, and product formation, while also accounting for the interrelationships between these factors. Both the Gompertz and logistic curves exhibit an S-shaped pattern, making them well-suited for modeling processes that involve an initial adaptation phase, followed by rapid growth, and eventually a slower phase as the system reaches saturation. Despite their visual and numerical similarities, these two models are fundamentally distinct.

In this study, the Gompertz model was found to be the most effective in explaining citric acid production and biomass formation, providing the highest goodness-of-fit (R^2) with μ_{max} 15 h^{-1}, P_{max} 24.97 g/L and R^2 0.995 as shown Table 4. when compared to other models such as the logistic and Monod models under all experimental conditions. This aligns with recent studies, such as that of Chaichol et al. (2023), who applied the Andrews model to describe substrate inhibition effects in Aspergillus species during citric acid fermentation. Their findings showed that the Andrews model offered a better fit than classical Monod kinetics, particularly at high glucose concentrations [17]. Similarly, Chaleewong et al. (2022) used a combined Contois-Gompertz hybrid model to more accurately capture biomass growth and product formation during batch fermentation of filamentous fungi, highlighting the significance of accounting for biomass-dependent substrate uptake [26]. These studies underline the evolving nature of kinetic modeling in fermentation research, demonstrating how hybrid or modified models often outperform traditional ones. By incorporating complex biological phenomena such as inhibition, biomass aggregation, and morphological changes, these advanced models provide a more comprehensive and accurate representation of fermentation dynamics.

Table 1. Results of *A. niger* cultivation experiments in batch bioreactor.

Fermentati on time (h)	Biomass (g cell/L)	Glucose Concentration (g glucose/L)	Citric acid concentration (g citric acid/L)
0	2.65	98.58	2.13
48	6.51	83.60	2.56
96	7.39	69.81	16.64
144	8.06	54.29	18.35
192	14.58	29.77	21.76
240	12.27	28.51	24.75
288	14.41	26.70	24.32
336	12.63	24.66	23.47
384	12.39	22.73	22.61

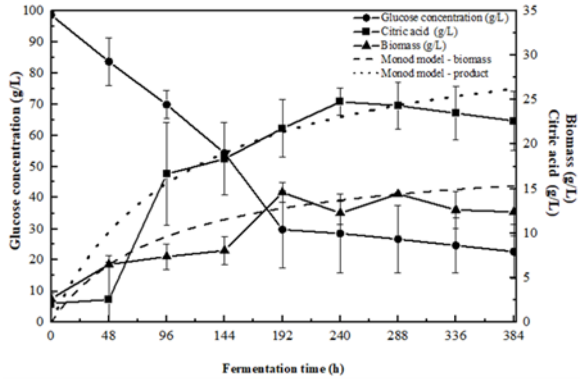
4.2 Substrate Consumption

For the consumption of sugar by *A. niger*, Table 1. shows a gradual decrease in the amount of residual sugar from an initial value of 98.58 g/L, which decreased to 22.73 g/L by the end of the fermentation, after 384 hours. The observed decrease was due to the sugar consumption by the microorganisms during fermentation. Our findings align with those reported by Amenaghawon & Aisien (2012) [26], who observed a similar reduction in the residual sugar content due to the formation of citric acid from the sugar consumption of corn starch hydrolysate *A. niger*.

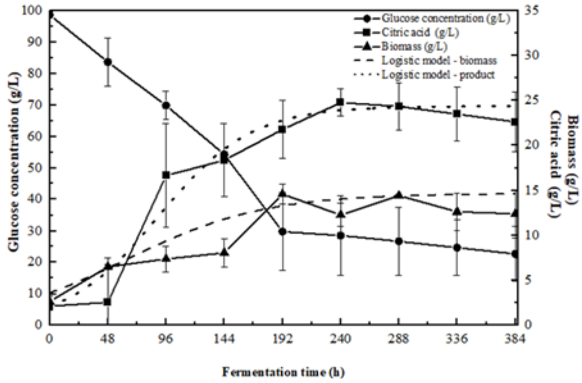
The parameters for sugar consumption, $Y_{x/s}$ and K_s, obtained from the Monod model were 0.0743 g cell / g glucose and 1.622 g/L, appeared in Tables 2 and 4 respectively.

4.3 Microbial Growth

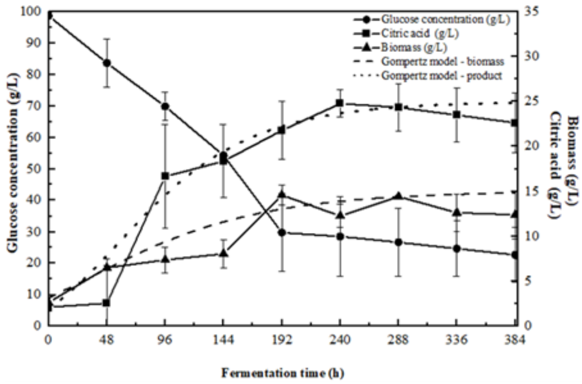
The growth of the fermented *A. niger* is shown in Table 1. It was observed that the concentration of *A. niger* cells increased from 2.65 g cell/L at the initial stage to 14.58 g cell/L after approximately 192 hours. After this point, the microbial concentration slightly decreased towards the end, with the final concentration being 12.39 g cell/L. The observed trend indicates that microbial growth occurred between 0 and 192 hours of fermentation, while growth slowed between 192 and 384 hours. The observed decrease is often due to the complete utilization of the substrate and may be caused by the high levels of citric acid are toxic to cells, when excessive citric acid accumulates [13], which could inhibit the activity of the fermenting microorganisms. The parameters for the growth of *A. niger*, μ_{max} and $Y_{x/s}$, as shown in Table 2., were 0.14 h^{-1} and 0.13 (g cell/g glucose), respectively. The experimental results are consistent with the findings of Amenaghawon & Aisien (2012) [26], who observed that the concentration of *A. niger* cells increased during the initial stages of fermentation, reaching a maximum value, after which the concentration decreased. The observed trend of decline is commonly attributed to the exhaustion of the substrate, which is consistent with Table 3 showing the kinetic parameters related to the microbial growth and citric acid production in the batch fermentation process. The microbial growth rate (r_x) started from 0.08 g cell/L h and gradually decreased, the sugar consumption rate (r_s) and citric acid production rate (r_p) also changed with the fermentation time, and the cell production rate (q_x), sugar consumption per cell (q_s), and citric acid production per cell (q_p) were also changed. These data can be used to improve and control the fermentation process to effectively increase the yield and reduce the cost of citric acid production from *A. niger*.



(a)



(b)



(c)

Fig. 3. Fitting of the (a) Monod model, (b) Logistic model and (c) Gompertz model to substrate, biomass and product concentrations by *A. niger* TISTR 2365 in 5 L bioreactor.

Table 2. Summary of *A. niger* TISTR 2365 growth kinetics data for batch cultivation in bioreactors.

μ_{max} (h^{-1})	$Y_{x/s}$ (g cell / g glucose)	$Y_{p/s}$ (g citric acid / g glucose)	$Y_{p/x}$ (g citric acid / g cell)	t_d (h)	Productivity (g/g/h)
0.14	0.13	0.27	2.10	398.26	0.15

4.4 Product Formation

Figure 3. shows the time profile of citric acid concentration during fermentation. It was observed that

citric acid production exhibited a nearly linear relationship with cell growth from the onset of fermentation up to approximately 192 hours, indicating that citric acid production is closely linked to microbial cell growth. The citric acid concentration increased steadily from 2.13 g/L to around 24.75 g/L, after which it plateaued and subsequently declined after 240 hours. These observations are consistent with those of Nadeem et al. (2010), who reported a similar decline in citric acid production after reaching a peak. According to Al-Sheri & Mostafa (2006) [28], Alvarez-Vasquez et al. (2000) [29], Arzumanov et al. (2000) [30], and Kristiansen & Sinclair (1978) [31], the decline in citric acid yield was attributed to reduced microbial growth in the fermentation after 96 hours, as citric acid formation is closely linked to microbial growth. Additionally, this decline may be due to inhibitory effects from high citric acid concentrations, reduced nitrogen levels in the fermentation medium, and the depletion of sugar sources. The parameters for citric acid production, specifically $Y_{p/x}$, were calculated and presented in Table 2, showing a value of 2.10 g of citric acid per gram of cells. High variability observed in Figure 3 can be attributed to several factors, including biological variability inherent to the microorganism, limitations in analytical techniques, and heterogeneity within the bioreactor environment. Biological variability, such as differences in spore germination, hyphal morphology, and pellet formation, can influence microbial growth and metabolite production across replicates. Furthermore, measurement techniques like dry weight determination and colorimetric assays may introduce significant experimental error, especially when sample preparation lacks consistency. In the earlier phase of this study, citric acid concentrations were analyzed using High-Performance Liquid Chromatography (HPLC). The results obtained from HPLC were found to be consistent with those obtained using the titration method, showing no significant differences in measurement accuracy. Therefore, to simplify the modeling and evaluation of the kinetic equations, the titration method was selected for subsequent analyses. This approach allows for efficient model validation while maintaining reliable accuracy in the assessment of citric acid production. Additionally, spatial gradients of nutrients and oxygen within the bioreactor could contribute to these discrepancies. To address these issues and enhance data reliability, high-performance liquid chromatography (HPLC) is recommended due to its superior sensitivity and specificity. HPLC provides more accurate quantification, reduces analytical errors, and improves reproducibility, thereby significantly enhancing the reliability of fermentation data. [26, 32]

Table 4. Values of estimated parameters using different models

Model	μ_{max} h^{-1}	P_{max} g/L	K_S g/L	R^2_b	R^2_p
Monod	19.08±0.98	33.83±14.54	93.98	0.97	0.92
Logistic	14.66±0.46	24.36±0.71	-	0.99	1.00
Gompertz	15.01±0.66	24.97±1.15	-	0.99	0.99

R^2_b: regression coefficient for fitted biomass concentration.

R^2_p: regression coefficient for fitted product concentration

Table 3. Kinetic parameters associated with biomass growth and citric acid production

r_x (g cell/L·h)	r_s (g glucose/L·h)	r_p (g product/L·h)	q_x (g cell/g glucose)	q_s (g glucose/g cell)	q_p (g product/g cell)
-	-	-	0.03	37.20	0.80
0.08	-0.31	0.01	0.08	12.84	0.39
0.02	-0.29	0.29	0.11	9.45	2.25
0.01	-0.32	0.04	0.15	6.74	2.28
0.14	-0.51	0.07	0.49	2.04	1.49
-0.05	-0.03	0.06	0.43	2.32	2.02
0.04	-0.04	-0.01	0.54	1.85	1.69
-0.04	-0.04	-0.02	0.51	1.95	1.86
-0.01	-0.04	-0.02	0.55	1.83	1.82

Note: q_x = biomass productivity (g/L h), q_s = sugar consumption rate (g/L h) and q_p = citric acid productivity (g/L h)

5 Conclusions

This study successfully demonstrated the use of Monod, Logistic, and Gompertz models to analyze microbial growth and citric acid production by *A. niger* in a batch fermentation system. The application of these models provided a deeper understanding of the kinetics involved in citric acid production, highlighting the importance of mathematical models in optimizing fermentation processes. Among the models tested, the Gompertz model showed the best fit to the experimental data, providing the highest goodness-of-fit (R^2) values. This model effectively captured the sigmoidal growth patterns and product formation kinetics, making it the most suitable for describing the dynamics of citric acid production under the given experimental conditions. The Logistic model, while also suitable, exhibited asymmetry compared to the more symmetric Gompertz model.

The findings confirm that microbial growth and citric acid production are closely linked, with the fermentation process following an initial exponential growth phase, followed by a deceleration period. The concentration of citric acid peaked at approximately 24.75 g/L, after which it declined due to the exhaustion of nutrients and accumulation of inhibitory products. These results are consistent with prior studies, which indicate that high citric acid concentrations and nutrient limitations can inhibit further microbial growth and acid production. Overall, the study emphasizes the importance of precise parameter estimation and model selection for optimizing industrial-scale fermentation processes. The insights gained from this research can aid in the efficient production of citric acid and similar metabolites, contributing to the advancement of biotechnological applications.

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