Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v45i1.63415

Validation of mathematical models as a tool for prediction of α-amylase production by *Coprinus comatus* in a low-cost culture medium

Luana Cristina Paludo[1*], Ana Paula Biz[1], Kamilla Marques Gonçalves[3], Christian Scapulatempo Strobel[4], Michelle Rossana Ferreira Vaz[2] and Michele Rigon Spier[1]

[1]Programa de Pós-Graduação em Engenharia de Alimentos, Departamento de Engenharia Química, Universidade Federal do Paraná, Av. Francisco Heráclito dos Santos, 100, 81531-980, Centro Politécnico, Curitiba, Paraná, Brazil. [2]Departamento de Engenharia Química, Instituto de Tecnologia, Universidade Federal do Pará, Belém, Pará, Brazil. [3]Departamento de Engenharia Química, Setor de Tecnologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil. [4]Departamento Engenharia Mecânica, Setor de Tecnologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil. *Author for correspondence. E-mail: luanacristinapaludo@gmail.com

ABSTRACT. Studying new microbial sources and evaluating the growth kinetics behavior to predict large-scale production become essential. Besides, research with low-cost culture media has gained interest due to the need to decrease enzyme production costs. The literature concerning the use of macromycetes as a source of amylases, and applying mathematical models to predict their behavior is scarce. In this study, four growth kinetics in different compositions of culture media were carried out: two of them using different concentrations of Low-Grade Flour (LGF), one wheat mill by-product and two synthetic ones. In the kinetics mathematical models were used to evaluate the bioprocesses and their scaling feasibility. Mathematical models, such as modified Logistic, modified Gompertz, Baranyi, and Roberts, and modified Luedeking-Piret, were applied to predict mycelial growth, enzyme production, and substrate consumption. The results showed the model's capability to predict mycelial growth (biomass) and substrate consumption (starch concentration). On the other hand, the mathematical models efficiently describe only the maximum enzymatic activity (α-amylase), not the process's other parameters, because of the complexity and diversity of biomolecules synthesized by *Coprinus comatus*. The wheat mill by-product was efficient as an ingredient in the culture media composition. Furthermore, it was concluded that B2 formulation (culture medium with 48.5 g/L of LGF) is the batch with the highest potential for the α-amylase production from *C. comatus* and the expansion of this process on an industrial scale. This study is important and valid for the scientific community since modeling studies for macromycetes are scarce in the literature.

Keywords: basidiomycetes; kinetics; biomass production; enzyme activity; substrate consumption.

Received on April 29, 2022.
Accepted on October 13, 2022.

Introduction

The α-amylases are significant given that they present several uses, mainly in the food industry, such as in the production of glucose syrup, fermentation, baking, and sweeteners (Iram et al., 2021). Likewise, α-amylases are used in biorefinery, medicine, paper production, and in the chemical industry - such as in alcohol production and starch hydrolysis (Aghaei, Mohammadbagheri, Hemasi, & Taghizadeh, 2022; Fasim, More, & More, 2021; Paris, Scheufele, Júnior, Guerreiro, & Hasan, 2012). These enzymes promote the hydrolysis of starch at random locations in the chain, originating compounds such as maltose, maltotriose, and limited dextrins (Paul, Lall, Jadhav, & Tiwari, 2017). Due to its variety of uses and compounds produced, this enzyme stands out in the enzymatic market.

Currently, this enzyme has been used as a substitute for emulsifiers in the food industry, mainly in bakery products, increasing the shelf life and freshness of bread, for instance (Ruan, Zhang, & Xu, 2022). There is an increasing interest of the world population in foods with fewer chemical products, such as food additives (Farias, Kawaguti, & Koblitz, 2021). The COVID-19 pandemic has progressively led to greater concern for immunity and health, causing consumers to seek organic and natural foods. This upsurge in demand is reflected in the enzyme market, with an estimated value of US$ 17.2 billion for the year 2027 (Ibrahim, Eid, & Amin, 2021). For the α-amylase market, this value should exceed US$ 2.7 billion by 2030.

Most industrial amylases are produced from microbial sources. Among microbial enzymes, fungi stand out due to their capacity to hydrolyze a variety of organic sources (Kumari, Sushil, Rani, Malik, & Avtar, 2019; Lim, Hazwani-Oslan, & Oslan, 2020). Although the high potential of macromycetes to bioconvert organic matter into other compounds, studies aiming at the production of amylases by macromycetes are limited (Frantz, Paludo, Stutz, & Spier, 2019; Uddin, Ferdous, Rahman, Roy, & Khan, 2013).

Coprinus comatus has a high potential to produce different biomolecules with high-added value and extracellular enzymes. This strain was quantitatively evaluated for amylase production only by Paludo et al. (2018) and Frantz et al. (2019). Although, there are no studies regarding the use of *Coprinus comatus* for enzymatic production in industrial scale, and no patents registered on "α-amylases produced by *C. comatus*" in the patent databases *Espacenet Patent Search* and *Google Patent*.

The cost of producing enzymes in industrial scale is high, and the culture medium used as a substrate is one of the main costs. Therefore, substitution by low-cost carbon sources is increasingly important in this market (Balakrishnan, Silva, Hwa, & Cremer, 2021; Porfirif, Milatich, Farruggia, & Romanini, 2016). The price of biomolecules can be more competitive by using low-cost substrates (Balakrishnan et al., 2021; Jujjavarapu & Dhagat, 2019). Taking it into consideration, Frantz et al. (2019) evaluated the centesimal and mineral composition of wheat mill by-products. The results showed that these by-products are a source of starch, protein, and minerals (calcium, potassium, and magnesium). One of these by-products is Low-Grade flour (LGF), usually used in wheat flour formulations and for animal feed production, and it has a low cost compared to commercialized flour. Frantz et al. (2019) also showed that LGF has the potential for amylase production by *Coprinus comatus*.

In this scenario, this work evaluated batches by applying mathematical modeling of biochemical processes to predict growth behavior, enzyme production, and substrate consumption in the medium and enable the scaling of the process. Considering that, mathematical modeling is defined as the attempt to express the operation of a reaction through mathematical equations. The application of this method is essential to understand the interactions in the bioprocess, as they relate the response variables to environmental conditions (such as temperature, pH, and substrate (Gomes, Fontana, Zielinski, Nogueira, & Spier, 2021; Coban & Demirci, 2016).

Material and methods

Microorganism

Coprinus comatus strain (A54ED3, Sisgen) was provided by the Mushroom Bioprocess Laboratory (LBC) of the Midwestern State University (UNICENTRO, Brazil). Reactivation, inoculation, and maintenance were performed as described by Frantz et al. (2019).

Inoculum

Three fragments (5x5 mm) of *C. comatus* strain were inoculated in 250 mL Erlenmeyer flasks containing 100 mL of 1% (m/v) aqueous suspension of Low-Grade Flour (LGF) (Frantz et al., 2019; Paludo et al., 2018) during 4 days, for mycelial growth. Subsequently, half percent of pellets (0.5 % m/v, wet weight) was inoculated in a 250 mL Erlenmeyer flask containing 100 mL of medium for 10 days. All processes were performed in a medium with pH 6.0, previously autoclaved at 121ºC for 15min. and incubated in a shaker (TECNAL, TE-424, BR) under orbital agitation at 120 rpm at 28 ± 2ºC.

Culture medium

The LGF was characterized by Frantz et al. (2019). This by-product has 13.24% of moisture, 2.68% of ash, 18.02% of proteins, and 63.91% of starch, with the presence of Ca^{2+}, P, Mg^{2+}, K^{+}, and Na^{+}. Culture samples were collected during the 240 h cultivation to evaluate the enzymatic activity, starch concentration, and biomass production. The carbon/nitrogen ratio of the batch of B1 by-product was 15.09/1, and for B2 it was 7.58/1. Table 1 presents the composition of the culture medium used in the experiments. The B2 medium was evaluated to test a concentration of C/N lower than B1, with the purpose to decrease the required amount of by-product used as substrate, as well as decrease residual starch at the end of the process. The culture medium was also evaluated for presenting α-amylase activity similar to those found in the optimal medium in the CCRD, in the tests performed by Frantz et al. (2019). The control culture media were prepared based on the composition of their respective media with by-products, CB1 with B1 and CB2 with B2.

Table 1. Compositions of the culture medium.

Batch	Culture medium composition
B1 (Batch 1)	105 g L^{-1} LGF*, 3.76 g L^{-1} urea, and 7.52 g L^{-1} KH_2PO_4
CB1 (Control Batch 1)	67.5 g L^{-1} P.A. pure starch, 3.76 g L^{-1} urea, 8.82 g L^{-1} K_2HPO_4, 0.12 g L^{-1} $CaCl_2$, 0.06 g L^{-1} NaCl, and 0.9 g L^{-1} $MgSO_4$.
B2 (Batch 2)	48.5 g L^{-1} LGF*, 4.0 g L^{-1} urea, and 8.0 g L^{-1} K_2HPO_4.
CB2 (Control Batch 2)	31 g L^{-1} P.A. pure starch, 4.0 g L^{-1} urea, 9.3 g L^{-1} K_2HPO_4, 0.06 g L^{-1} $CaCl_2$, 0.075 g L^{-1} NaCl, and 0.4 g L^{-1} $MgSO_4$.

*LGF – Low-Grade Flour; B1: medium with by-product; CB1: medium with concentrations of starch and minerals similar to B1, considering the composition described by Frantz et al. (2019); B2: medium with by-product; CB2: medium with concentrations similar to B2.

Analytical methods

Biomass concentration was measured as dry cell mass, and starch concentration was quantified using the AOAC methodology (996.11, 1995). Enzyme assay (α-amylase) was determined by the colorimetric method proposed by Fuwa (1954) and adapted by Paludo et al. (2018).

Determination of kinetics parameters and conversion factors

Calculations of doubling time (D_t), conversion factors ($Y_{X/S}$, $Y_{P/S}$, and $Y_{P/X}$), biomass productivity (Y_X), enzyme productivity (Y_P), and specific maximum microbial growth rate (μ_{max}) were performed for all the tested batches considering the data obtained during cultivation. Some parameters were calculated using the method proposed by Le Duy and Zajic (Schmidell, Lima, Aquarone, & Borzani, 2021). The equations are shown in Table 2.

Table 2. Equations to calculate the parameters of evaluated kinetics.

Kinetic Parameters and Conversion Factors			
Doubling time		$D_t = \frac{\ln 2}{\mu_{máx}}$	
Conversion Factors	$Y_{\frac{X}{S}} = -\frac{X_f - X_0}{S_0 - S_f}$	$Y_{\frac{P}{S}} = -\frac{P_f - P_0}{S_0 - S_f}$	$Y_{\frac{P}{X}} = \frac{P_f - P_0}{X - X_0}$
Productivity	$Y_X = \frac{X_f - X_0}{t_t}$		$Y_P = \frac{P_f - P_0}{t_t}$
Specific maximum mycelial growth rate		$\mu_{máx} = \frac{\ln X - \ln X_0}{t_f - t_0}$	

X_0 - initial biomass (g L^{-1}); X_f - final biomass (g L^{-1}); t_t – Total cultivation time (h); S_0 – initial substrate (g L^{-1}); S_f – final substrate (g L^{-1}); P_0 – initial product (U L^{-1}); P_f – final product (U L^{-1}); D_t – Doubling time (h); $Y_{X/S}$ - Substrate to biomass conversion factor (g_x g_b^{-1}); $Y_{P/S}$ - Substrate to product conversion factor (U g_s^{-1}); $Y_{P/X}$ - Biomass to product conversion factor (U g_x^{-1}); Y_x - Biomass productivity (g L^{-1} h^{-1}); Y_p - Enzyme productivity (U L^{-1} h^{-1}); μmax - Specific maximum mycelial growth rate (1 h^{-1}).

Mathematical modeling in bioprocess kinetics

Different mathematical models (Table 3) were tested to evaluate the experimental data obtained in the growth kinetics as previously done by Gomes et al. (2021) and Spier, Letti, Woiciechowski, and Soccol (2009). All models were calculated using OriginPro 8.5 (OriginLab, Massachusetts, USA, 2018) and Microfit® (Siqueira, Carvalho, Mendes, & Shiosaki, 2014). The coefficient of determination (R^2) and the Root mean square error (RMSE) were used to determine the proper fit. The changes made to the models are detailed in the respective references. The present authors only converted the positive sign from 1 to negative in the Gompertz model (Cheng, Demirci, Catchmark, & Puri, 2010), which was applied to predict biomass growth. During our analysis, we realized that the original model was not able to predict biomass growth. The biomass growth predicted by the original model always occurred in an accelerated way, not predicting the experimental data. After the change, the model was able to predict the behavior of this type of microorganism.

Results and discussion

Kinetics for mycelial growth, enzymatic production, and substrate consumption

The first assay set was carried out to evaluate the enzymatic kinetics in two different cultures media and their respective control media (CB1 and CB2). Figure 1 presents the kinetics performed for the evaluation of mycelial growth (biomass), enzyme production (α-amylase activity), and substrate consumption (starch concentration).

Table 3. Mathematical models used to evaluate mycelial growth, enzyme production, and substrate consumption.

Models	Equation	Calculated parameters	Reference
Mycelial growth (biomass production)			
Modified Gompertz	$X(t) = X_f \exp\left\{-exp\left[\frac{\mu_{max} e}{X_f}(\lambda - t) - 1\right]\right\}$	X_f, μ_{max}	Cheng et al. (2010) with changes by the authors
Modified Logistic	$X(t) = \frac{X_0 exp(\mu_{max} t)}{1 - \left(\frac{X_0}{X_f}\right)(1 - exp(\mu_{max} t))}$	μ_{max}, X_f	Ramprakash and Muthukumar (2015)
Baranyi and Roberts	$X(t) = X_0 + \mu_{max}\left(x + \left(\frac{1}{X_f}\right) log\left(\frac{1 + exp^{\left(-X_f(t-\lambda)\right)}}{1 + exp^{X_f \lambda}}\right)\right)$	X_f, μ_{max}, lag (λ)	Siqueira (2014)
Enzyme production (α-amylase activity)			
Modified Luedeking and Piret	$P(t) = P_0 + \alpha A(t) + \beta B(t)$ $A(t) = P_0\left(\frac{exp(\mu_{max} t)}{1 - \frac{P_0}{P_{max}}(1 - exp(\mu_{max} t))}\right)$ $B(t) = \frac{P_{max}}{\mu_{max}} ln\left(1 - \frac{P_0}{P_{max}}(1 - exp(\mu_{max} t))\right)$	P_{max}, μ_{max}, α, β	Tarafdar et al. (2021); Gomes et al. (2021)
Modified Gompertz	$P(t) = P_{max} \exp\left\{-exp\left[\frac{Y_P e}{P_{max}}(\Lambda - t) + 1\right]\right\}$	P_{max}, (Λ)	Mahdinia et al. (2019)
Substrate consumption (Starch concentration)			
Modified Logistic	$S(t) = \frac{S_0}{1 + exp(\mu_{max}(\tau - t))}$	μ_{max}, τ	Mahdinia et al. (2019)

P – product (enzyme); t – cultivation time; α – empirical constant dependent on microbial growth; β – empirical constant non-dependent on microbial growth; P_0 – product initial; (t); P_{max} – maximum activity enzyme; Y_P – productivity enzyme; λ – lag phase; adaptation phase for enzyme production (Λ); X_0 – initial biomass; X_f – final biomass; S_0 – initial substrate; e – Euler constant; 2.71; μ_{max} – the specific maximum rate of substrate degradation; τ – time of maximum substrate degradation.

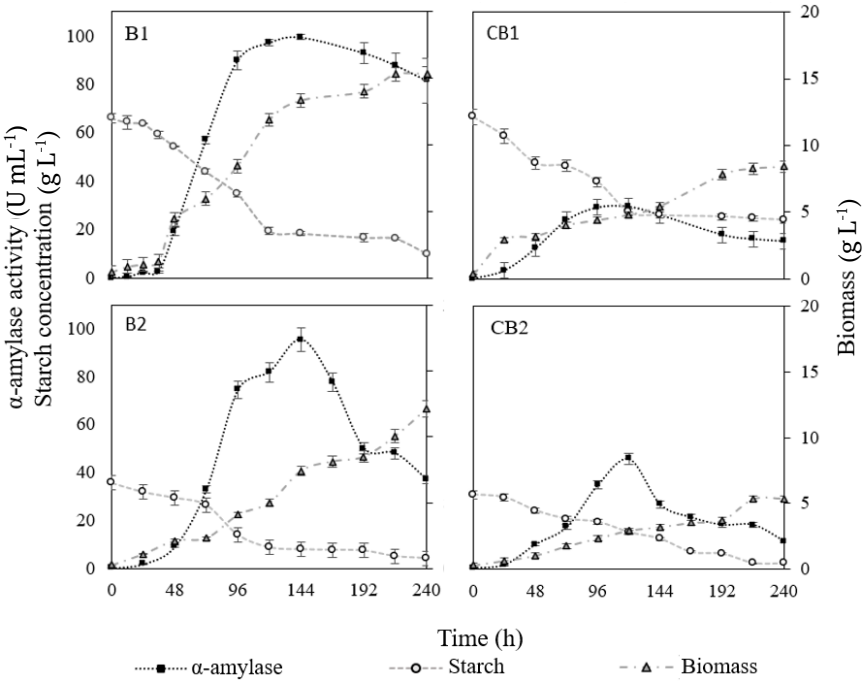

Figure 1. Cultures for α-amylase synthesis by *Coprinus comatus*, with fixed inoculation rate conditions (0.5 % m/v), initial pH (6.83), incubation temperature (28 ± 2°C) at 120 rpm in an orbital shaker.

The batches B1 and B2 with LGF as substrate showed the highest biomass production, above 12 g L^{-1}, and the control batches (CB1 and CB2) showed concentrations below 9 g L^{-1}. This difference may be linked to the biomolecule's availability in the medium due to the complexity of LGF composition compared to the synthetic medium. According to Frantz et al. (2019), the wheat mill by-product is rich in proteins, lipids, fiber, calcium, magnesium, and phosphorus, among other complex compounds essential for mycelial growth. Studies report the importance of highly complex culture media to increase macromycetes growth (Frantz et al., 2019;

Adebayo-Tayo & Ugwu, 2011). The starch and proteins presented in LGF are sources of power, carbon, nitrogen, amino acids, and essential compounds for the metabolism of living creatures. From these facts, it is possible to understand the difference between batches with by-products and those with a synthetic medium.

Furthermore, by analyzing the behavior of the strain in the batches, it is possible to assert that enzyme production is associated with mycelial growth. This behavior was also observed in studies by Frantz et al. (2019).

The starch hydrolysis at the maximum enzyme activity (144h, 99.51 ± 1.11 U mL^{-1}) was 72 % in batch B1, while batch CB1 hydrolyzed 59.24 % of the starch at peak enzymatic activity (120h, 29.77 ± 1.23 U mL^{-1}). In batch B2, α-amylase hydrolyzed 42.03 % of the starch in 144h at the maximum activity (95.51 ± 2.70 U mL^{-1}), while at CB2, the starch hydrolysis was only 36.05 % in 120h at the peak (46.20 ± 1.27 U mL^{-1}). Batch B2 and CB2 showed lower starch hydrolysis over time. On the other hand, they presented lower residual starch concentration than B1 and CB1. This is important for the feasibility of the industrial process, as there is less waste for disposal and treatment.

Batch B2 and CB2 showed lower starch hydrolysis over time, on the other hand, showed lower residual starch concentration compared to B1 and CB1. This is important for the viability of the industrial process, as there is less waste for disposal and treatment.

The control batches showed an enzymatic activity peak in a shorter time than their respective batches with by-products, at 120h for CB1 and CB2, compared to 144h for B1 and B2. On the other hand, control batches had a lower enzymatic activity, with CB1 3.34-fold lower than B1 and CB2 2.06-fold lower than the activity obtained in B2. The maximum activity of B1 and B2 occurred at 144h of cultivation; in addition, the B1 batch showed enzymatic activity 9% higher than B2, but no statistical difference by Tukey's test ($p > 0.05$) between the samples.

From this fact, it is possible to infer that batch B2 is the most interesting for future processes. Also, since the LGF amount required for batch B2 production is 53.81% lower than for B1, the cost of production will be reduced. Therefore, B2 can be considered a low-cost batch for α-amylase production from *C. comatus*.

From the results obtained in B1, CB1, B2, and CB2, the kinetic parameters of the bioprocess were calculated for the α-amylase production, μ_{max}, D_t, enzyme production rate, biomass, and conversion factors ($Y_{X/S}$, $Y_{P/S}$, and $Y_{P/X}$), which are shown in Table 4.

Table 4. Kinetic parameters of the bioprocess obtained in cultivation performed in batches B1, CB1, B2, and CB2.

Parameters	B1	CB1	B2	CB2	Paludo et al. (2018)	Frantz et al. (2019)
Biomass (g L^{-1})	15.35	8.41	12.12	5.37	0.77	3.78
μ_{max} (1 h^{-1})	0.0146	0.0134	0.0159	0.0122	0.03	0.02
P_{max} (U mL^{-1})	99.51 ± 1.11 (144h)	29.77 ± 1.23 (120h)	95.51 ± 2.70 (144h)	46.20 ± 1.27 (120h)	5.84 (48h)	62.70 (136h)
D_t (h)*	47.42	51.88	43.73	57.00	22.13	38.61
Y_P (U L^{-1} h^{-1})	339.16	64.51	153.60	46.61	-	-
Y_X (g L^{-1} h^{-1})	0.062	0.033	0.049	0.021	0.01	0.01
$Y_{P/S}$ (U gds^{-1})'	1448.5	364.69	1175.5	391.58	323.00	814.28
$Y_{X/S}$ (g gds^{-1})	0.265	0.19	0.3781	0.1777	0.06	0.06
$Y_{P/X}$ (U gdb^{-1})''	5465.1	1919.3	3109.4	2203.1	5260.02	12542.34

'gds – g of a substrate; ''gdb – g of biomass; *D_t – doubling time; μ_{max} – specific maximum mycelial growth rate; Y_P – hourly enzyme productivity; Y_X – hourly biomass productivity; $Y_{P/S}$ –productivity of the substrate; $Y_{X/S}$ – biomass productivity with the substrate; $Y_{P/X}$ – product (enzyme) productivity to biomass; P_{max} – maximum activity enzyme.

The batches with LGF medium, B1 and B2, showed a shorter doubling time than their control batches, 4.46h (CB1) and 13.27h (CB2), respectively. However, for this strain, these values were higher than the other studies reported in the literature as presented in Table 2. The specific growth rate showed the same behavior, but the values of the current study were lower than those reported in the literature. Therefore, the difference could be related to biomass production or nutrient concentration in the medium. These can increase or inhibit the specific maximum growth rate. Batch B1 and B2 showed a higher specific growth rate due to the significant nutrient concentration in the medium. Vaz et al. (2011) also observed that the culture medium with the highest nutrient concentration increased μ_{max} and other parameters.

In terms of productivity (Y_P and Y_X) the growth kinetics with by-products (B1 and B2) were proper for α-amylase and biomass production. The enzyme production rate (Table 1) for the evaluated batches was 339.165 U L^{-1} h^{-1} for B1 and 153.6 U L^{-1} h^{-1} for B2. On the other hand, lower values were observed for synthetic medium batches (CB1 and CB2): 64.51 U L^{-1} h^{-1} for CB1, and 46.61 U L^{-1} h^{-1} for CB2, being 5.26 and 3.29-fold lower than B1 and B2, respectively. It is worth mentioning that when evaluating the productivity (Y_p) at the peak of

enzymatic activity, it was observed that B1 and B2 presented productivity of 413.82 U L^{-1} h^{-1} and 396.80 U L^{-1} h^{-1}, respectively, not differing statistically from each other by the Tukey test ($p > 0.05$). By-product media also showed better Y_x than synthetic media. This difference in batch productivity can be explained by the composition of by-product media concerning synthetic media. As previously mentioned, the LGF has in its composition, compounds that may have induced a better production of biomass and enzyme, which resulted in more expressive production factors.

The substrate to biomass conversion factor ($Y_{X/S}$) obtained values of 1.87 and 2.33-fold higher when comparing B1 with BC1 and B2 with BC2, respectively. The biomass to product conversion factor ($Y_{P/X}$) also showed this characteristic, with B1 2.84-fold higher than CB1, and B2 1.41-fold bigger than CB2. It also can be seen when the substrate to product conversion factor was analyzed. The substrate to product conversion factor ($Y_{P/S}$) was 1448.5 U gds^{-1} (B1) and 1175.5 U gds^{-1} (B2), while CB1 was 364.69 U gds^{-1} and CB1 was 391.58 U gds^{-1}. These results mean that for each gram of substrate consumed, there is a production of more than 1,000 U for batches with LGF compared to a range lower than 400 U for the synthetic medium.

The differences between by-product and synthetic media batches reinforce the idea that macromycetes adapt and develop better in complex and rich medium. This type of macro-organism is usually present in nature and is considered decomposers, symbionts, and even parasites (Niego et al., 2023). This may be one of the reasons that this by-product has more satisfactorily induced mycelial growth and α-amylase production.

Conversion factors and yield parameters are important when choosing the strain used, operating parameters, and cultivation medium in order to reduce production costs (Frantz et al., 2019). It is estimated that about 30 to 40% of the cost involved in enzyme production is related to the culture medium (Joo & Chang, 2005; Kumar & Takagi, 1999). Therefore, there is an increasing search for agro-industrial by-products that can be substrates for enzyme production to reduce the total production cost (Paul et al., 2017). The LGF proved to be suitable for α-amylases production by *C. comatus* compared to the synthetic medium. Besides, the use of LGF in culture media is favorable because of the low cost.

Mycelial growth modeling (Biomass)

The Logistic model modified by Um, Wang, and Yu (2005) was used to predict the maximum specific growth rate (μ_{max}). In this study, the modified Gompertz model (Cheng et al., 2010) was modified by the authors for the mycelial growth of macromycetes to predict X_f and Y_x. The mathematical model proposed by Baranyi and Roberts (1994) allowed the determination of μ_{max} and λ (lag phase of growth) parameters. All models related mycelial growth with the cultivation time and maximum biomass production (X_f). Data obtained are shown in Table 5.

The μ_{max} predicted by the modified Logistic model varied from 0.023 to 0.040 1 h^{-1}. The values were similar to those found by Paludo et al. (2018) (μ_{max} = 0.032 1 h^{-1}) and by Frantz et al. (2019) (μ_{max} = 0.02 1 h^{-1}). The μ_{max} obtained by the Logistic model and the Baranyi and Roberts mathematical model was higher than the experimental ones. The same also happened with the modified Gompertz model, probably because they did not consider substrate consumption.

Table 5. Predicted and calculated parameters for the modified Logistic model, modified Gompertz model, and Baranyi and Roberts model to assess mycelial growth.

Model	Batches	μ_{max} (1 h^{-1})	X_f (g L^{-1})	λ (h)	R^2	RMSE
Modified Logistic	B1	0.040	14.88	-	0.988	0.641
	CB1	0.025	7.98	-	0.919	1.535
	B2	0.029	11.07	-	0.966	0.759
	CB2	0.023	5.32	-	0.946	0.407
Modified Gompertz	B1	0.340	15.33	-	0.991	0.545
	CB1	0.092	8.58	-	0.951	0.547
	B2	0.155	11.58	-	0.985	0.451
	CB2	0.064	5.33	-	0.970	0.287
Baranyi and Roberts	B1	0.072	16.85	28.00	0.930	1.735
	CB1	0.037	9.08	12.28	0.950	0.887
	B2	0.054	11.99	57.98	0.987	0.452
	CB2	0.021	5.34	19.01	0.975	0.261

The modified Logistic model predicted an X_f for B1 with a variation of 3.05% concerning experimental data, and the predicted value of CB1 had a variation of 6.08% compared to the observed results. For batch B2, which uses 48.5 g L^{-1} of by-product, the predicted and experimental values of X_f differed by 8.68%, and CB2 showed a deviation of 0.68%. Although the modified Logistic model did not consider the substrate consumption in the kinetics bioprocess, this model did not vary by more than 10% compared to the experimental results; besides, all R^2 and RMSE were higher than 0.9 and lower than 0.2, respectively. Therefore, the modified Logistic model is mainly suitable for predicting the *C. comatus* mycelial growth.

The X_f values predicted by the modified Gompertz model had a lower variation than experimental data (Figure 2). It was 0.09% for B1, 2.04% for CB1, 4.44% for B2, and 0.53% for CB2.

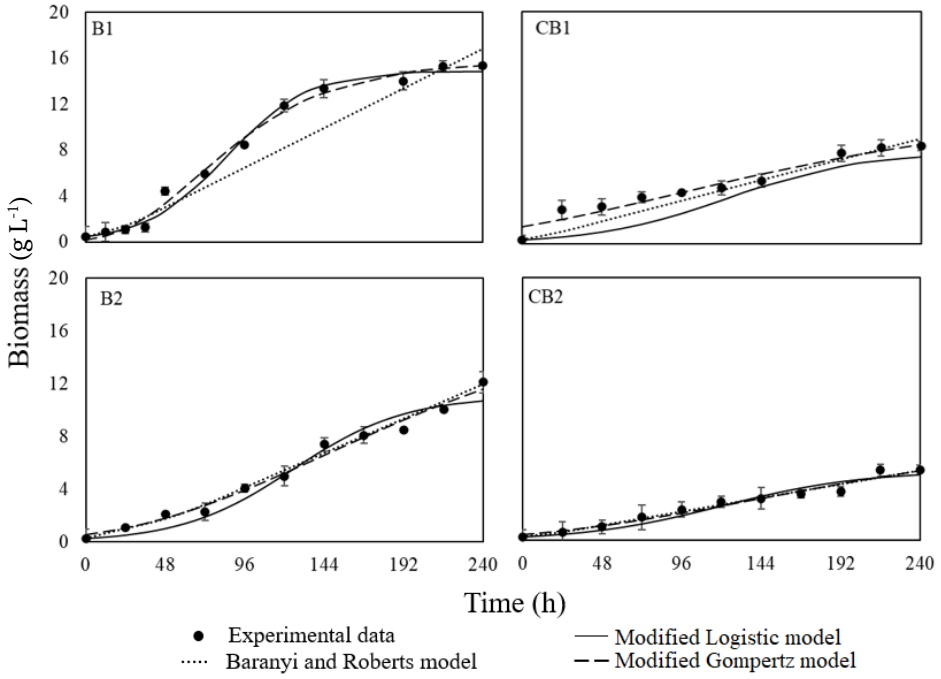

Figure 2. Observed biomass production (mycelial growth) and fitted models (modified Logistic, modified Gompertz and Baranyi and Roberts).

Baranyi and Roberts model predicted final biomass with a difference lower than 10% when compared to the experimental data. Batch B1 had an X_f of 16.85 g L^{-1}, which was 9.77% higher than the experimental value (15.35 g L^{-1}). The CB1 control batch also obtained a similar difference on the B1 batch, with 9.69% more biomass obtained by the mathematical model compared to the kinetic. The batch B2, with by-products, showed an experimentally higher biomass production than the predicted value by the model (1.09%), and the CB2 control batch achieved 0.37% more biomass than predicted by the model.

The lag phase (λ) obtained by the Baranyi and Roberts model for batch B1 was approximately 28h, while for CB1 it was 12.28h. Batch B2 had a λ of 57.98h, and CB2 had a lag phase of 19.01h. These values were very similar to those found experimentally. The λ of the control kinetics was lower than the batches with by-products, probably due to the use of a less complex synthetic medium than the LGF, which reduced the required time to adapt the strain to the environment.

The modified Logistic model could predict mycelial growth with an $R^2 > 0.9$ and RMSE < 2 for all batches. The modified Gompertz model was also significantly correlated to the experimental data ($R^2 > 0.951$ and RMSE < 1). The R^2 and RMSE obtained for Baranyi and Roberts showed that the mathematical model can also satisfactorily predict biomass production by *C. comatus*. However, the most appropriate model to predict biomass growth was the modified Gompertz model.

Mathematical modeling for enzyme production (α-amylase activity)

The modified Luedeking-Piret model estimates the α and β values, indicating whether production formation depends or not on mycelial growth. When $\alpha \neq 0$ and $\beta = 0$, enzyme formation is associated with mycelial growth. On the other hand, if $\alpha = 0$ and $\beta \neq 0$, product formation is not associated with mycelial

growth (Germec et al., 2019; Mohammad, Badr-Eldin, El-Tayeb, & El-Rahman, 1995). The parameters (α, β, and μ_{max}) calculated by the model are presented in Table 6.

The second mathematical model assessed for product formation was the modified Gompertz (Mu et al., 2005; van Ginkel, Sung, & Lay, 2001; Lin & Lay, 2004) which relates the enzymatic activity to cultivation time, and predicts the adaptation phase for enzyme production (ʌ) for enzyme production in each batch as well. The results, R^2 and RMSE for each mathematical model application, are shown in Table 6.

Table 6. Model parameters for the kinetics of product formation (α-amylase activity).

Model	Batch	P_{max} (U mL^{-1})	Λ (h)	α	β	μ_{max} (1 h^{-1})	R^2	RMSE
Modified Luedeking-Piret	B1	92.99	-	6045.65	0	0.076	0.973	5.71
	CB1	29.14	-	3458.00	0.0012	0.08	0.748	5.91
	B2	95.49	-	7856.00	0	0.062	0.707	25.72
	CB2	42.58	-	7896.00	0	0.063	0.522	18.16
Modified Gompertz	B1	96.67	40.32	-	-	-	0.985	7.28
	CB1	27.00	24.82	-	-	-	0.740	7.34
	B2	91.93	69.17	-	-	-	0.735	28.57
	CB2	44.70	36.67	-	-	-	0.524	17.60

The P_{max} experimental value and the data obtained by the modified Luedeking-Piret model presented a difference; it was 6.54% for B1, 2.12% for CB1, 0.02% for B2, and 7.83% for CB2. The P_{max} obtained in the experiments, B1 was 99.50 U mL^{-1}, while the modified Gompertz Model predicted a maximum of 96.67 U mL^{-1} (2.85% deviation). Meanwhile, the predicted productivity in BC1 and the experimental value showed a deviation of 9.32%. Batch B2 showed a deviation of 3.20%, and CB2 presented a value of 3.75%. However, all predicted values had a deviation of less than 10% of the experimentally achieved.

The adaptation phase for enzyme production (ʌ) values obtained by the modified Gompertz model differed from the experimental data. Also, considering the D_t obtained experimentally, a parameter directly linked to the ʌ, it is possible to prove that this model cannot satisfactorily predict the behavior of macromycetes. On the other hand, the values obtained for P_{max} were comparable to the experimental ones.

The association between biomass formation and biomolecule synthesis was reported by Osadolor, Nair, Lennartsson, and Taherzadeh (2017), where α was 3.189 and β was 0.077 1 h^{-1}. The authors observed that pellet formation was associated with ethanol production when the *Neurospora intermedia* fungi strain was used. This behavior was also observed in the present study, where α was higher than β (β value was approximately 0 for all batches). The significance of α compared to β presented that for these batches, the production of the enzyme is associated with the growth of *C. comatus,* - a behavior experimentally verified (Figure 3).

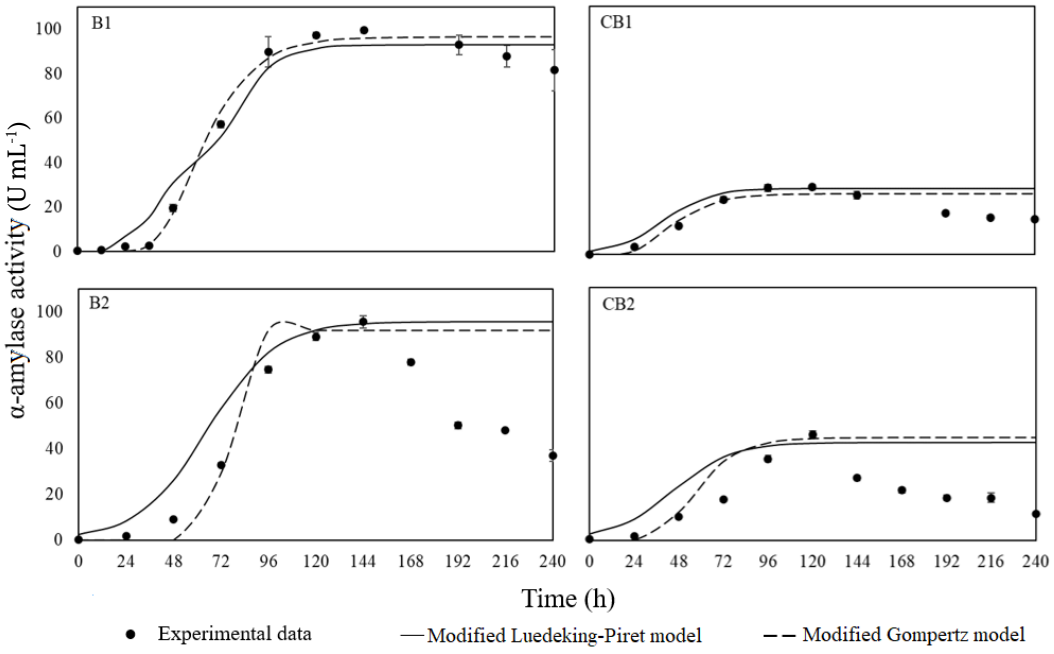

Figure 3. Observed α-amylase activity and fitted models (modified Luedeking-Piret and modified Gompertz).

The maximum specific growth rate (μ_{max}) predicted by the modified Luedeking-Piret model for the batches indicated higher values than those found experimentally. This may be related to the model not considering the other biomolecules synthesized together with the biomass.

The R^2 and RMSE values showed that the mathematical models used to predict the product formation could not efficiently describe all batches. It can be related to the mathematical model's inability to predict the enzyme activity decrease over time. In this process the models did not consider substrate degradation and the fundamental enzyme/substrate ratio. However, in industrial-scale enzyme production, the process is stopped when the enzyme reaches its maximum activity; thus, the mathematical models would be efficient in predicting the process as both can predict the maximum activity of α-amylase (Figure 3).

Mathematical Modeling for substrate consumption (starch concentration)

The Logistic model was originally developed to assess mycelial growth (Zwietering, Jongenburger, Rombouts, Van, & Riet, 1990). However, it was modified by Kargi (2009) for substrate consumption (Mahdinia, Mamouri, Puri, Demirci, & Berenjian, 2019). These modifications allowed to calculate the maximum specific rate of substrate consumption (μ) and the time of maximum substrate consumption (τ). The results for the model's parameters are shown in Table 7.

Table 7. Parameters were obtained by the modified Logistic model for substrate consumption (starch concentration).

Model	Batch	μ	τ (h)	R^2	RMSE
Modified Logistic	B1	0.022	109.54	0.932	0.545
	CB1	0.011	143.48	0.821	6.318
	B2	0.025	98.31	0.938	3.110
	CB2	0.022	120.29	0.982	1.334

The specific rate of substrate consumption (μ) for the evaluated batches ranged from 0.011 1 h^{-1} (CB1) to 0.025 1 h^{-1} (B2). The maximum consumption time (τ) of the substrate ranged from 98.31h (B2) to 143.48h (CB1). B1 and B2 showed a shorter consumption time than their control batches, CB1 and CB2, respectively. This fact suggests that the higher the enzymatic activity in the batch process, B1 and B2, the shorter the substrate consumption time. The consumption time can be observed in Figure 4, and it is possible to notice that there is no significant difference between the experimental data and the one predicted by the mathematical model. The R^2 and RMSE values obtained from the modified Logistic model also demonstrated that the model could be used to describe the process of all batches.

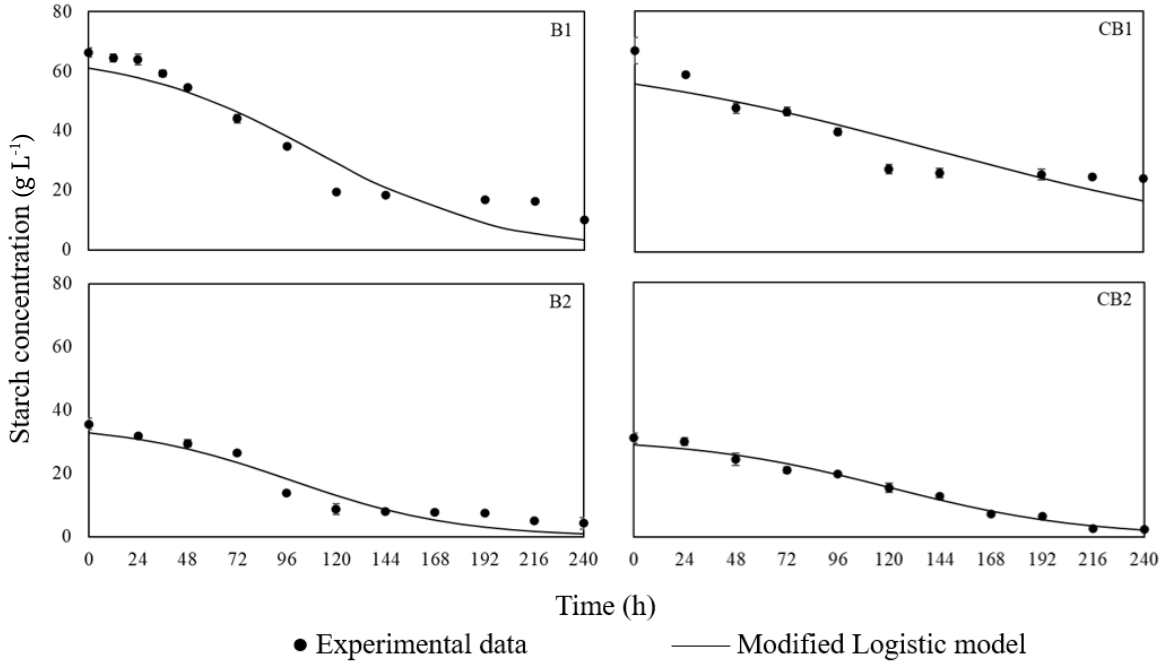

Figure 4. Observed starch concentration (substrate consumption) and fitted model (modified Logistic).

Some models presented in the literature to evaluate kinetic parameters were developed to analyze different living beings, such as the Logistic and Gompertz models that initially evaluated human population growth and their mortality rate. Other models, such as the Luederking-Piret and Baranyi and Roberts models, evaluated kinetics with bacteria. These mathematical models, over time, were adapted and tested to be used in different microorganisms, most of which have duplication time and growth behavior different from macromycetes. While there have been mathematical models for bacteria and yeast since 1949, for instance, the model proposed by Monod (Dette, Melas, Pepelyshev, & Strigul, 2003), the interest in modeling filamentous fungi, has only increased in recent decades (Dantigny & Panagou, 2013). Macromycetes studies are even more recent and limited.

The facts presented may explain the problems found in applying the mathematical models in macromycetes kinetics since the delay in the doubling time and the hydrolysis of the components are directly related to mycelial growth, substrate consumption, and biomolecule synthesis. On the other hand, that does not make these mathematical models unsuitable, as it can satisfactorily predict the parameters required to scale up the bioprocess, as showed in the present work. In this way the work was important for enabling and evaluating different models in bioprocesses with *C. comatus* in the production of α-amylase. In addition, enzyme production was improved using wheat mill by-products compared to synthetic medium, which demonstrates the requirement to evaluate other by-products as substrate. More studies need to be done to develop or improve mathematical models to effectively describe the kinetics with macromycetes.

Conclusion

This study used mathematical models to predict mycelial growth, α-amylase synthesis by *Coprinus comatus*, and substrate hydrolysis in 4 different media compositions. The three models applied to mycelial growth could predict the X_f values with a difference of less than 10% compared to the experimental data. Evaluating α-amylase synthesis, the modified Gompertz model and the Luederking-Piret model also predicted P_{max} with a difference of less than 10% from the experimental value. The modified Logistic mathematical model was important for evaluating the substrate hydrolysis kinetics because it could predict the range of highest substrate consumption.

Acknowledgements

The authors are grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted (No. 88882.381649/2019-01).

References

Adebayo-Tayo, B. C., & Ugwu, E. E. (2011). Influence of different nutrient sources on exopolysaccharide production and biomass yield by submerged culture of *Trametes versicolor* and *Coprinus* sp. *AU Journal of Technology*, *15*(2), 63-69.

Aghaei, H., Mohammadbagheri, Z., Hemasi, A., & Taghizadeh, A. (2022). Efficient hydrolysis of starch by α-amylase immobilized on cloisite 30B and modified forms of cloisite 30B by adsorption and covalent methods. *Food Chemistry*, *373*(Part A), 1-6. DOI: https://doi.org/10.1016/j.foodchem.2021.131425

Association of Official Analytical Chemists. (1995). *Official Methods of Analysis* (16th ed.). Washington, DC.

Balakrishnan, R., Silva, R. T., Hwa, T., & Cremer, J. (2021). Suboptimal resource allocation in changing environments constrains response and growth in bacteria. *Molecular Systems Biology*, *17*(12), 1-13. DOI: https://doi.org/10.15252/msb.202110597

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*, *23*(3-4), 277-294. DOI: https://doi.org/10.1016/0168-1605(94)90157-0

Cheng, K. C., Demirci, A., Catchmark, J. M., & Puri, V. M. (2010). Modeling of pullulan fermentation by using a color variant strain of *Aureobasidium pullulans*. *Journal of Food Engineering*, *98*(3), 353-359. DOI: https://doi.org/10.1016/j.jfoodeng.2010.01.011

Coban, H. B., & Demirci, A. (2016). Enhancement and modeling of microparticle-added *Rhizopus oryzae* lactic acid production. *Bioprocess and Biosystems Engineering*, *39*(2), 323-330. DOI: https://doi.org/10.1007/s00449-015-1518-0

Dantigny, P., & Panagou, E. (2013). *Predictive Mycology* (1th ed.). NewYork, NY: Nova Publishers.

Dette, H., Melas, V. B., Pepelyshev, A., & Strigul, N. (2003). Efficient design of experiments in the Monod model. *Journal of the Royal Statistical Society Series B: Statistical Methodology*, *65*(3), 725-742. DOI:https://doi.org/10.1111/1467-9868.00412

Farias, T. C., Kawaguti, H. Y., & Koblitz, M. G. B. (2021). Microbial amylolytic enzymes in foods: Technological importance of the *Bacillus genus. Biocatalysis and Agricultural Biotechnology*, *35*, 1-6. DOI: https://doi.org/10.1016/j.bcab.2021.102054

Fasim, A., More, V. S., & More, S. S. (2021). Large-scale production of enzymes for biotechnology uses. *Current Opinion in Biotechnology*, *69*, 68-76. DOI: https://doi.org/10.1016/j.copbio.2020.12.002

Frantz, S. C., Paludo, L. C., Stutz, H., & Spier, M. R. (2019). Production of amylases from *Coprinus comatus* under submerged culture using wheat-milling by-products: Optimization, kinetic parameters, partial purification, and characterization. *Biocatalysis and Agricultural Biotechnology*, *17*, 82-92. DOI: https://doi.org/10.1016/j.bcab.2018.11.004

Fuwa, H. A. (1954). A new method for micro determination of amylase activity using amylase as the substrate. *The Journal of Biochemistry*, *41*, 583-603. DOI: https://doi.org/10.1093/oxfordjournals.jbchem.a126476

Germec, M., Gürler, H. N., Ozcan, A., Erkan, S. B., Karahalil, E., & Turhan, I. (2020). Medium optimization and kinetic modeling for the production of *Aspergillus niger* inulinase. *Bioprocess and Biosystems Engineering*, *43*, 217–232. DOI: https://doi.org/10.1007/s00449-019-02219-1

Gomes, T. A., Fontana, É., Zielinski, A. A. F., Nogueira, A., & Spier, M. R. (2021). Optimizing the growth-associated β-galactosidase production by probiotic *Lactobacillus reuteri* B-14171: experimental design, culture medium volume increase, and cell growth modeling. *Scientia Plena*, *17*(4), 1-15. DOI: https://doi.org/10.14808/sci.plena.2021.040203

Ibrahim, N. A., Eid, B. M., & Amin, H. A. (2021*).* Sustainable textile finishing processes and pollution control based on enzyme technology. In *Green chemistry for sustainable textiles* (p. 385-415). DOI: https://doi.org/10.1016/B978-0-323-85204-3.00018-X

Iram, N., Shakir, H. A., Khan, M., Ali, S., Anwer, A., Saeed, S., & Qazi, J. I. (2021). Statistical optimization of amylase production using grape fruit peels in submerged fermentation. *Acta Scientiarum. Technology*, *43*(1), 1-11. DOI: https://doi.org/10.4025/actascitechnol.v43i1.50538

Joo, H. S., & Chang, C. S. (2005). Oxidant and SDS-stable alkaline protease from a halo-tolerant *Bacillus clausii* I-52: enhanced production and simple purification. *Journal of Applied Microbiology*, *98*(2), 491-497. DOI: https://doi.org/10.1111/j.1365-2672.2004.02464.x

Jujjavarapu, S. E., & Dhagat, S. (2019). Evolutionary trends in industrial production of α-amylase. *Recent Patents on Biotechnology*, *13*(1), 4-18. DOI: https://doi.org/10.2174/2211550107666180816093436

Kargi, F. (2009). Re-interpretation of the logistic equation for batch microbial growth in relation to Monod kinetics. *Letters in Applied Microbiology*, *48*(4), 398-401. DOI: https://doi.org/10.1111/j.1472-765X.2008.02537.x

Kumar, C. G., & Takagi, H. (1999). Microbial alkaline proteases: from a bioindustrial viewpoint. *Biotechnology Advances*, *17*(7), 561-94. DOI: https://doi.org/10.1016/s0734-9750(99)00027-0

Kumari, N., Sushil, Rani, B., Malik, K., & Avtar, R. (2019). Microbial amylases: An overview on recent advancement. *Journal of Entomology and Zoology Studies*, *7*(1), 198-205.

Lim, S. J., Hazwani-Oslan, S., & Oslan, S. N. (2020). Purification and characterisation of thermostable α-amylases from microbial sources. *Bioresources*, *15*(1), 2005-2009.

Lin, C.-Y., & Lay, C. H. (2004). Effects of carbonate and phosphate concentrations on hydrogen production using anaerobic sewage sludge microflora. *International Journal of Hydrogen Energy*, *29*(3), 275-281. DOI: https://doi.org/10.1016/j.ijhydene.2003.07.002

Mahdinia, E., Mamouri, S. J., Puri, V. M., Demirci, A., & Berenjian, A. (2019). Modeling of vitamin K (Menaquinoe-7) fermentation by *Bacillus subtilis natto* in biofilm reactors. *Biocatalysis and Agricultural Biotechnology*, *17*, 196-202. DOI: https://doi.org/10.1016/j.bcab.2018.11.022

Mohammad, F. H. A., Badr-Eldin, S. M, El-Tayeb, O. M., & El-Rahman, O. A. (1995). Polysaccharide production by *Aureobasidium pullulans* III. The influence of initial sucrose concentration on batch kinetics. *Biomass Bioenergy*, *8*(1995), 121-129.

Mu, Y., Wang, G., & Yu, H-Q. (2005). Kinetic modeling of batch hydrogen production process by mixed anaerobic cultures. *Bioresource Technology, 97*(11), 1302-1307. DOI: https://doi.org/10.1016/j.biortech.2005.05.014

Niego, A. G. T., Rapior, S., Thongklang, N., Raspé, O., Hyde, K. D., & Mortimer, P. (2023). Reviewing the contributions of macrofungi to forest ecosystem processes and services. *Fungal Biology Reviews*, 44. DOI: https://doi.org/10.1016/j.fbr.2022.11.002

Osadolor, O. A., Nair, R. B., Lennartsson, P. R., & Taherzadeh, M. J. (2017). Empirical and experimental determination of kinetics of pellet growth in filamentous fungi: A case study using *Neurospora intermedia*. *Biochemical Engineering Journal*, *124*, 115-121. DOI: https://doi.org/10.1016/j.bej.2017.05.012

Paludo, L. C., Frantz, S. C., Ançay, Jr., R., Stutz, H., Dantas, T. L. P., & Spier, M. R. (2018). Optimization, kinetic and bioprocess parameters of amylases production from *Coprinus comatus* under submerged culture using starch-based simple medium: Partial enzyme characterization. *Biocatalysis and Agricultural Biotechnology*, *16*, 529-537. DOI: https://doi.org/10.1016/j.bcab.2018.09.022

Paris, L. D., Scheufele, F. B., Júnior, A. T., Guerreiro, T. L., & Hasan, S. D. M. (2012). Produção de complexos enzimáticos por *A. niger* a partir de soja por fermentação em estado sólido. *Acta Scientiarum. Technology*, *34*(2), 193-200. DOI: https://doi.org/10.4025/actascitechnol.v34i2.9447

Paul, J. S., Lall, B. M., Jadhav, S. K., & Tiwari, K. L. (2017). Parameter´s optimization and kinetics study of α-amylase enzyme of *Bacillus* sp. MB6 isolated from vegetable waste. *Process Biochemistry*, *52*, 123-129. DOI: https://doi.org/10.1016/j.procbio.2016.10.005

Porfirif, M. C., Milatich, E. J., Farruggia, B. M., & Romanini, D. (2016). Production of alpha-amylase from *Aspergillus oryzae* for several industrial applications in a single step. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1022*, 87-92. DOI: https://doi.org/10.1016/j.jchromb.2016.04.015

Ramprakash, B., & Muthukumar, K. (2015). Comparative study on the performance of various pretreatment and hydrolysis methods to produce biohydrogen using *Enterobacter aerogenes* RM 08 from rice mill wastewater. *International Journal of Hydrogen Energy*, *40*(30), 9106-9112. DOI: https://doi.org/10.1016/j.ijhydene.2015.05.027

Ruan, Y., Zhang, R., & Xu Y. (2022). Directed evolution of maltogenic amylase from *Bacillus licheniformis* R-53: Enhancing activity and thermostability improves bread quality and extends shelf life. *Food Chemistry*, *381*, 1-6. DOI: https://doi.org/10.1016/j.foodchem.2022.132222

Schmidell, W., Lima, U. A., Aquarone, E., & Borzani, W. (2021). *Biotecnologia Industrial. Engenharia bioquímica* (2th ed.). São Paulo, SP: Blucher.

Siqueira, A. A., Carvalho, P. G. S., Mendes, M. L. M, & Shiosaki, R. K. (2014). MicroFit: um software gratuito para desenvolvimento e ajuste de modelos matemáticos de crescimento bacteriano. *Brazilian Journal of Food Technology*, *17*(4), 329-339. DOI: https://doi.org/10.1590/1981-6723.6414

Spier, M. R., Letti, L. A. J., Woiciechowski, A. L., & Soccol, C. R. (2009). A simplified model for *A. niger* FS3 growth during phytase formation in solid state fermentation. *Brazilian Archives of Biology and Technology, 52*(Spe.), 151-158. DOI: https://doi.org/10.1590/S1516-89132009000700020

Tarafdar, A., Sirohi, R., Guar, V. K., Kumar, S., Sharma, P., Varjani, S., ... Sim, S. J. (2021). Engineering interventions in enzyme production: Lab to industrial scale. *Bioresource Technology*, *326*, 1-7. DOI: https://doi.org/10.1016/j.biortech.2021.124771

Uddin, M. R., Ferdous, K., Rahman, M. A., Roy, A. K., & Khan, M. R. (2013). Amylatic Activity of *Agaricus* and Moulds to produce Bioethanol. *Chemical Engineering and Science*, *1*(1), 7-11. DOI: https://doi.org/10.12691/ces-1-1-2

van Ginkel, S., Sung, S., & Lay, J. J. (2001). Biohydrogen production as a function of pH and substrate concentration. *Environmental Science & Technology*, *35*, 4726-4730.

Vaz, M. R. F., França, R. L., Andrade, S. S., Sousa Jr., F. C., Santos, E. S., Martins, D. R. A., & Macedo, G. R. (2011). Influence of culture medium on the production of eif antigen from *Leishmania chagasi* in recombinant *Escherichia coli*. *Brazilian Journal of Microbiology*, *42*, 1390-1396.

Zwietering, MH, Jongenburger, I., Rombouts, FM, Van, T., & Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*, *56*(6), 1875-1881. DOI: https://doi.org/10.1128/aem.56.6.1875-1881.1990