Dynamic Modeling of Tannase Production from *Bacillus cereus:* A Framework Simulation based on Fed Batch Strategy

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Abstract. Tannase enzyme is a metabolite of great interest in the industry. Typical examples of its use can be found in wines and beer production, beverage and juice fruits clarification and leather production. However, tannase production on an industrial scale is limited to the operation batch mode. For this reason, its production is low and severe limitations take the place of being carried on a large scale. To improve production, this research proposes an operation strategy based on Fed-batch mode. The kinetic constants were taken from the literature to simulate trends obtained through a Feed-batch mode of operation. One of the most important findings of this research focuses on increasing tannase production with found values of 0.380 U/g. The latter indicates that tannase production could be almost twice the concentration obtained with the traditional batch mode (0.1900 U/g). Results obtained in this research may be promising for the enzyme production industry. Using computational techniques, it is possible to identify an improvement without investing in excessive experimentation and resources.

1. Introduction

Nowadays, biotechnology has been experimenting with scientific and technological advances. The latter helps industries and environment growth and renewal through the modification of established landscapes. One of the methods that have been implemented in the sectors is the use of microorganisms for enzyme production, which have been relieving chemical processes. Enzymes began to manufacture beneficial products for human consumption around 2000 years through microorganisms for bread production and rice saccharification before the fermented "koji" production [1]. Based on the above, around 5500 enzymes have been discovered today [1]. *Bacillus cereus* is a mesophilic and neutrophilic microorganism [2]. It is one of the great enhancers in microbial fermentation used to produce extracellular enzymes [3].

The tannase enzyme, also known as tannin acyl-hydrolase, is an esterase responsible for the prototype galloyl ester bonds [4]. These ester bonds are also present in hydrolyzable tannins and complex tannins [5], a set of impermeable polyphenolic compounds with different molecular weights [6]. Additionally, tannase is an enzyme produced by bacteria, fungi, and yeast. By hydrolyzing tannic acid through tannase, the release of gallic acid, glucose and some galloyl esters is obtained. Tannase is applied in food and beverage processes. Its main applications are focused on the production of instant

tea, acid and acorn liquor [7]. This enzyme is also used in the production of beers and wines to clarify some fruit juices and in the leather industry, among others [8].

Tannase has been extracted from microorganisms, plants and animals [9-10]. However, its large-scale application has been limited due to its low throughput and high production costs [11-13]. Even so, its production is based on batch mode.

Tannase is obtained through a closed system, so its production is limited to the dynamic concentration of the culture medium present in the bioreactor [14]. As the culture medium is consumed, tannase is simultaneously produced in the bioreactor. Considering the above, once all the culture medium has been exhausted, the bioreactor must be disassembled, sterilized, and loaded again with a fresh culture medium to restart a new fermentation process. The batch mode leads to fermentation and labor slow times that could significantly affect the tannase enzyme's global productivity. A production strategy that could avoid these process downtimes consists of the feed-batch mode [13]. A fresh culture medium is added at intervals of fermentation to prevent the biochemical process deceleration and simultaneously maintain tannase production for a longer time than obtained in a batch mode. That is why through this research, computational bioprocess modeling is proposed using a Feed-batch strategy. To carry out an operating conditions analysis, the Latter allows obtaining better tannase productivity from *Bacillus cereus*. It could mean a saving in time and budget for enzyme sector industries [12,15].

2. Methodology

In this research, an unstructured non-segregated mathematical model simulates tannic acid utilization, microbial growth rate, and tannase production. The volume of Feed-batch equipment depends on the fresh culture medium feeding and speed of culture medium depletion extraction, in such a way the dynamic volume for tannase production could be modeled using Eq (1):

$$\frac{dV}{dt} = F - F_{out} \tag{1}$$

The above means that *Bacillus cereus* cells growing in the bioreactor are also influenced by the fresh medium feed rate, in such a way that Biomass dynamic growth on dilution rate effects is modeled by Eq. (2):

$$\frac{dXV}{dt} = \mu XV - F_{out}X \tag{2}$$

X is the dynamic state concentration of *Bacillus cereus,* and μ is the microbial growth rate. To consider the simultaneous impact of the substrate and biomass concentration on the microbial growth rate in Fed-batch mode, μ is calculated from Eq. (3), according to the Monod model [16] coupled to logistic growth [14]:

$$\mu = \mu_{max}\left(\frac{S}{k_s+S}\right)\left(1-\frac{X}{X_m}\right) \tag{3}$$

S in the substrate concentration (tannic acid), μ_{max}, k_s and X_m are model kinetic constants. For tannase production, tannic acid is used as a carbon source and precursor. That is why tannic acid is used for microbial growth, product formation, and intracellular maintenance reactions, according to Eq. (4):

$$\frac{dSV}{dt} = FS_i - q_sXV - F_{out}S \tag{4}$$

Where S_i is the tannic acid concentration and q_s is the rate of substrate consumption and is calculated with Eq. (5):

$$q_s = m\mu + nq_p + m_s \quad (5)$$

Where q_p is the product formation rate, m_s is a coefficient that takes into account the substrate consumption effect due to cell maintenance. The parameters m and n are constants of the model.

In a bioreactor operated in Fed-batch mode, the tannase dynamic state concentration can be modeled considering the fresh medium feed rate and the product formation rate, resulting in Eq. (6):

$$\frac{dPV}{dt} = q_p XV - F_{out}P \quad (6)$$

Furthermore, previous studies [12] suggest tannase production as a metabolite indirectly associated with microbial growth. Tannase specific rate formation can be modeled in a bioreactor operated in Fed-batch mode using the Luedeking-Pired model [14] from Eq. (7):

$$qp = (\alpha + \beta\mu) \quad (7)$$

The Runge-Kutta 45 numerical method was used with Matlab R2017b software to solve the differential equations proposed in this research. The initial conditions are defined in Table 1.

Table 1. Initial conditions for tannase production Feed-batch simulations

Parameter	Value	Units
$\boldsymbol{S_0}$	58.00	mg/g
$\boldsymbol{X_0}$	0.438	g/g
$\boldsymbol{P_0}$	0.000	U/g
$\boldsymbol{V_0}$	6.000	L

The simulation is initially programmed on batch mode fermentation during the first 60 hours so that no fresh medium is fed to the bioreactor (F = Fout = 0) in this period. The above, to obtain the dynamic behavior of *Bacillus cereus* cells without feeding fresh medium effects. After 60 hours, the first tannic acid feed pulse is added as a culture medium, at a rate of 1.0 L/h and a tannic acid concentration (S_i) of 58 mg/g. The proposed production strategy to increase the tannase production from *Bacillus cereus* ends with fresh medium feeding from 96 to 180 hours of fermentation at a rate of 2 L/h (S_i = 58 mg / g).

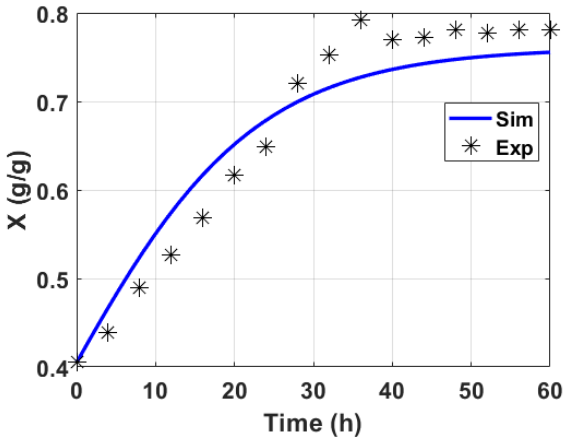
3. Results and Discussions

This research proposes an operation strategy in Fed-batch mode with programmed substrate feeding pulses for the tannase enzyme production to improve tannase production. In this way, it is possible to carry out a simulation analysis that considers the best conditions of a feed-batch operation model to analyze the increase in tannase production from *Bacillus cereus* microorganisms. Before performing the Fed-batch mode simulations, a calibration procedure was implemented to perform a manual adjustment of the model kinetic constants. The above, to achieve the best fitting between the experimental data taken from the bibliography and the simulated data. The results are shown in Table 2 and Fig. 1:

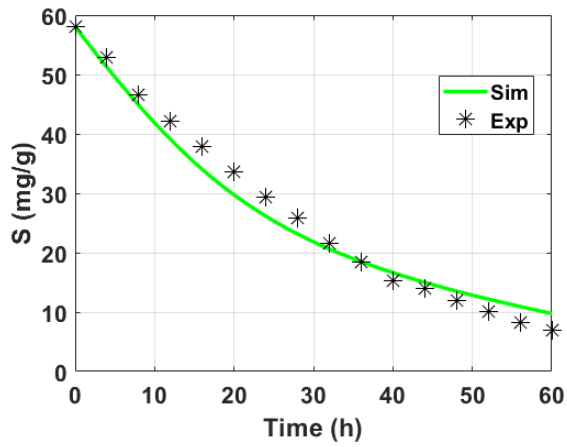
Table 2. Kinetic parameter fitted for Feed-batch tannase production from *Bacillus cereus*.

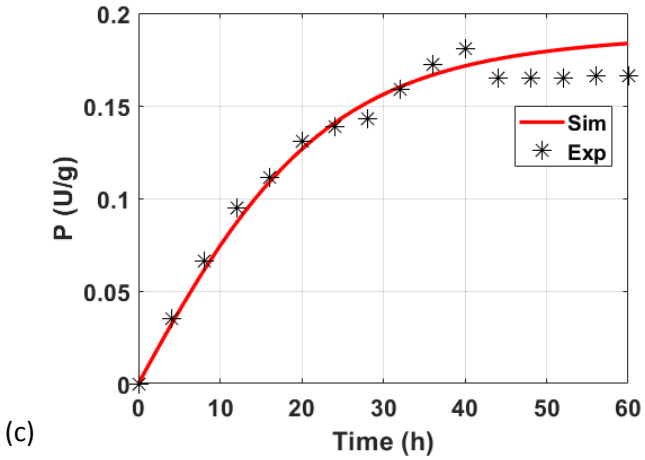
Parameter	Value	Unit
μ_{max}	0.08580	h^{-1}
k_s	1.50000	mg/g
β	0.50580	[-]
α	0.00016	[-]
m	319.820	g/g
n	-434.540	g/U
m_s	0.40000	[-]
X_m	0.76100	g/g

(a)



(b)





(c)

Figure 1. Model calibration for tannase production from experimental data [14]. Marks (experiments) and Solid lines (simulated data). (a) Biomass, (c) Substrate and (c) Product. (F = Fout = 0).

According to the results obtained (Fig. 1), there is an acceptable precision of the mathematical model planned in this investigation to simulate the microbial growth of *Bacillus cereus*, the consumption of tannic acid as a precursor and carbon source and the formation of the tannase enzyme.

It is worth mentioning that the model proposed in this research includes the effects of biomass and substrate in the production of tannase. In such a way that the impacts of biomass inhibition on all reaction rates have been considered in the model through the logistic model.

Once the mathematical model's adjustment of kinetic constants was carried out, the implementation was extended to Fed-batch mode's operation strategy, proposed in this research. The results obtained for biomass, tannase and use of tannic acid are observed in Fig. 1. The analysis was carried out in both batch and feed-batch modes to determine their dynamic state effects on tannase production. It can be seen in Fig. 2 (a) that biomass in batch mode reaches a maximum concentration of 0.76 g/g at 60 hours, and there is a decrease in feed-batch mode, obtaining a concentration of 0.60 g/g at 180 hours. It can also be seen in Fig. 2 (b) that the substrate in batch mode starts with a concentration of 58 mg/g, and as the fermentation process passes, it decreases until it reaches a concentration of 10 mg/g at 60 hours. After first substrate pulse feeding, an increase in its concentration is observed until a mean value of 33 mg/g. The latter is because the rate of substrate addition is higher than the tannic acid uptake rate. However, the above is a temporary phenomenon since once the consumption rate exceeds the dilution rate, the substrate drops drastically to minimum values.

According to the feed-batch production strategy proposed in this research, the second tannic acid feed pulse was carried out at 96 hours of fermentation at a rate of 2 L/h and 58 mg/g of tannic acid. It is observed in Fig. 2 (b) that the mathematical model responds satisfactorily to the mentioned stimulus. However, as the substrate uptake rate is higher, all the substrate fed to the bioreactor is instantly consumed by *Bacillus cereus* cells. The preceding could be considered a starting point for future optimization investigations that allow knowing the ideal feed rate to maximize the tannase concentration in a bioreactor operated in Fed-batch mode. Contrary to batch mode, better assimilation of tannic acid by the biomass is observed, with minimum values of 0.18 mg/g at 180 hours.

According to the equations proposed in this research, tannase formation is regulated with the bioreactor's biomass and substrate concentrations. The above, argued in previous experimental data [14], highlights the importance of maintenance factors associated with tannase production. In Fig. 2 (c), a tannase concentration of 0.190 U/g is observed in batch mode at 60 hours. Interestingly, with the Fed-batch strategy proposed in this research, a simulated concentration of 0.380 U/g at 180 hours is achieved, which indicates that the tannase concentration could increase almost twice with the values obtained at batch mode. Similar results have been reported in other investigations. Such is the case of

[17], who evaluated the effectiveness of a Feed-batch process for lysine (ε-PL) amino acid production using *Bacillus cereus* growing on glucose pulses. They found 85 mg/L of ε-PL at 96 hours of fermentation in batch mode in the same report. However, with the addition of glucose pulses in Fed-batch mode, they managed to identify a concentration of the mentioned product of 422 mg/L.

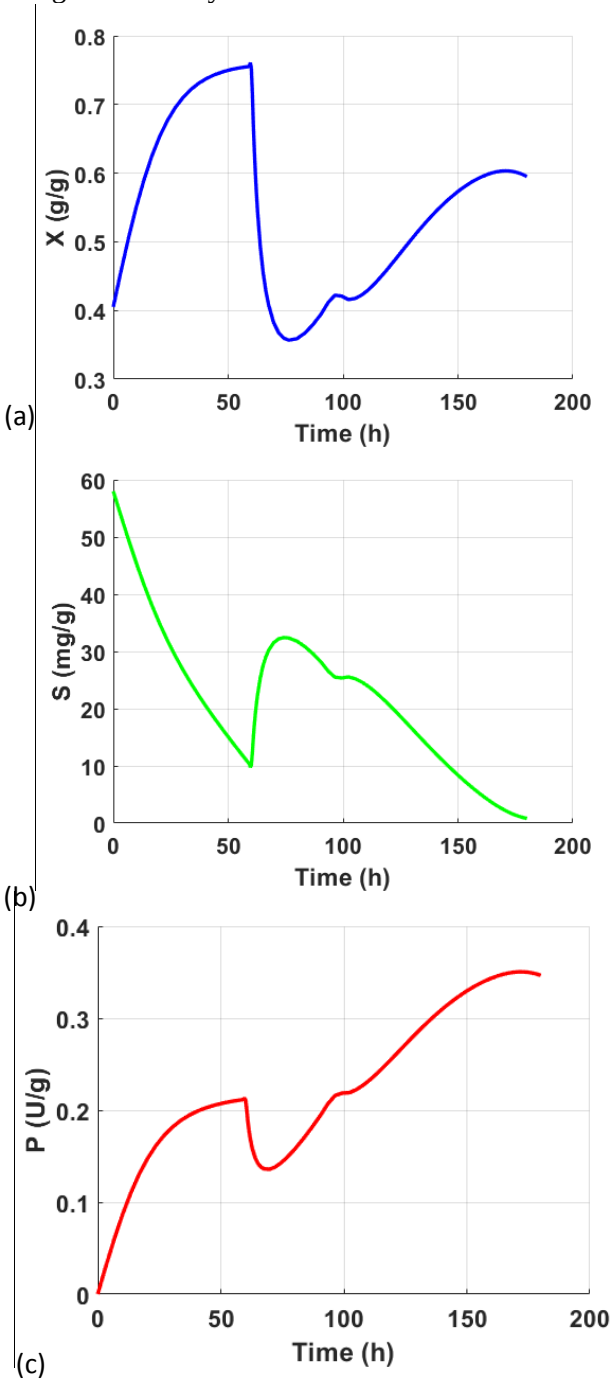


Figure 2. Simulation results for Feed-batch tannase production. (a) Biomass, (c) Substrate and (c) Product.

According to Eq. (1), unlike a bioreactor in batch mode, the operating volume is influenced by the feed flow so that it is not constant during fermentation in Fed-batch mode. According to the results

obtained in this investigation (Fig. 3), a volume of almost 50 liters is reached at 96 hours of operation at the end of the first feeding pulse of 1 L/h. Once the fermentation is finished in Fed-batch mode at 180 hours, the operating volume exceeds 200 liters. According to the above, the design volume of the bioreactor requires a size greater than 200 liters. Considering a tank with a maximum carrying capacity of 75% [16], a bioreactor size of 270 liters of total capacity is required to implement the Fed-batch strategy proposed in this research.

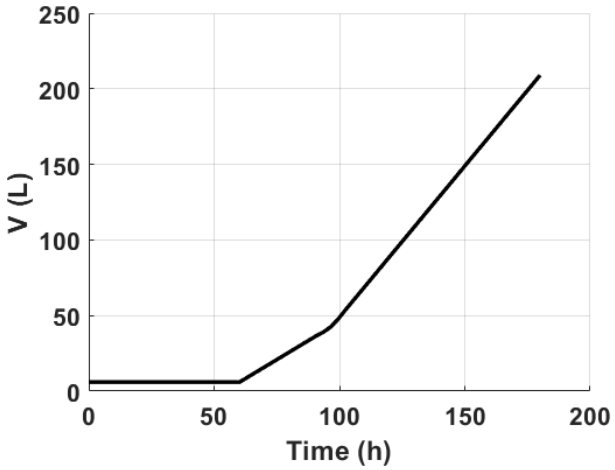


Figure 3. Bioreactor volume profile simulated for Feed-batch tannase production.

4. Conclusions

This research proposed an operation strategy in Fed-batch mode with programmed substrate feeding pulses to improve production. The Fed-batch mode adopted in this investigation controls the kinetics of tannase production. The above is constituted as a starting point for future optimization research. The results obtained in this research may be promising for the enzyme production industry. It is possible to identify improvements to an industrial process through computational techniques without investing in excessive experimentation and resources.

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