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Cellulase Production from *Trichoderma harzianum*: a Framework Modeling for Evaluating Different Operating Mode Strategies

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Abstract. The cellulase enzyme has been used in essential applications in the textile industry and the formulation of some medicines. Therefore, a mathematical model based on previous reports is proposed for cellulase enzyme enhancement. First, the kinetic constants were taken from the literature. Then, two operating strategies were carried out to maximize cellulase production using 10g/L of cellulose and 0.4g/L of cells as a first strategy using a batch mode. Alternatively, a second operating mode is simulated using continuous cellulose feeding. Finally, a comparative analysis was made for determining the best operating mode related to maximal cellulase production. Interestingly, the continuous mode shows a high cellulase concentration lasting a shorter time than the batch mode to avoid inhibiting enzyme production. One of the most important findings of this work focuses on increasing cellulase production with values of 1800 FPT/L simulating the continuous mode. The latter suggests that cellulase production could be double the concentration obtained with the traditional batch mode (800FPU/L). Results obtained in this research may be promising for the enzyme production industry. Using simulation techniques, it is possible to determine an enhancement without requiring excessive experimentation and resources.

1. Introduction

The search for new renewable energies has motivated the study of biofuels from lignocellulose biomass such as wheat straw, sorghum, or sugarcane bagasse, from which fermentable reducing sugars are obtained through hydrolysis enzymatic, including cellulose [1]. Likewise, the biotechnological production of ligninolytic enzymes has specific economic and technical advantages. For example, they can be produced on a large scale with predictable yield and potential to catalyze many particular reactions and the ease of being separated from products formed [2]. Thus, the production of cellulases has received great credit for its numerous applications in the industrial field. In addition, however, they have generated a significant impact in obtaining fermentable sugars, forming essential final products such as bioethanol.

In the industry, the most widely used system to produce enzymes is the batch mode, which consists of a bioreactor that is fed at the beginning of the process with nutrients and microorganisms, allowing their growth for the necessary time until the desired product is obtained without adding or extracting the material from the bioreactor [3]. The reactor generally consists of a stirred tank. The container must be well mixed so that the compositions are the same in any part of the reactor at a given time.

However, the mode generates disadvantages that range from low yields to inherent downtime between charges. In addition, its operating cost can be relatively high since it has a non-stationary state that implies control of the process (product is more difficult to obtain). The operating mode is implemented to feed a bioreactor with nutritive substances to control the cell death period. In addition, it has significant benefits such as improvements in the bioprocessing of metabolites since control strategies in a steady state can be set up and do not manage a defined cultivation time [4-6]. It should be noted that these operating methods are beneficial when a high cell density is required in the initiation stage of the process that involves the uptake of a high nutrient.

This work aims to evaluate different cellulose production strategies through mathematical models based on the biochemical behavior of the microorganism *Trichoderma harzianum* using cellulose as a limiting substrate. The latter is focused on finding an efficient bioprocess control. Therefore, the batch and continuous mode comparison will be made to observe and identify the most efficient strategy in obtaining cellulase.

2. Methodology

Cellulose is the most abundant renewable carbon source on earth [7]. It is the main component of the cell wall of most plants. It is synthesized through photosynthesis, and it is characterized by being resistant to fermentation. The agricultural residues are very rich in cellulose, hemicellulose, and lignin. Cellulose can be used as substrates for cultivating filamentous fungi capable of producing extracellular enzymes with cellulase activities, with critical industrial applications, such as the hydrolysis of lignocellulosic biomass for ethanol production [8]. In this work, an unstructured non-segregated mathematical model is used to simulates cellulase production.

The *Trichoderma harzianum* growing in the bioreactor is modeled considering the batch or continuous operating mode as shown in Eq. (1):

$$\frac{dX_T}{dt} = \mu \left(1 - \frac{X_T}{X_{Tm}}\right) X_T - k_d X_T - D X_T \quad (1)$$

X_T is the dynamic state concentration of *Trichoderma harzianum*, and μ is the microbial growth rate. D is the dilution rate for continuous operating mode (for a batch process, $D = 0$). To consider the simultaneous impact of the substrate and biomass concentration on the microbial growth rate, μ is calculated from Eq. (2), according to the Monod model [9]:

$$\mu = \mu_{max} \left(\frac{C}{k_c + C} \right) \quad (2)$$

According to the kinetic model k_d is the cell death rate and is calculated based on Eq. (3):

$$k_d = k_{dmax} \left(\frac{C}{k_{di} + C} \right) \quad (3)$$

C in the cellulose concentration, μ_{max} , k_c , X_{Tm} and k_{di} are model kinetic constants. Due to the presence of cellulose, a part of total cells (X_{cell}) is activated to produce cellulase. Therefore, a mathematical expression [3] is used to consider the activated cell concentration responsible for cellulase production based on Eq. (4):

$$\frac{dX_{cell}}{dt} = \mu_{cell} \left(1 - \frac{X_{cell}}{X_{cellm}}\right) X_T - k_{dcell} X_{cell} - D X_T \quad (4)$$

Where X_{cell} is the active cell concentration. X_{cellm} is the maximal concentration of active cells. k_{dcell} and μ_{cell} are the specific dead and active cell growth rates, respectively. The latter is calculated according to Eq. (5):

$$\mu_{cell} = \mu_{cellmax} \left(\frac{C}{k_{cell} + C} \right) \quad (5)$$

$\mu_{cellmax}$ and k_{cell} are model kinetic constants for active cells producing cellulase. Cellulose is used for microbial growth and cellulase formation, according to Eq. (5):

$$\frac{dC}{dt} = -\alpha \left[\mu \left(1 - \frac{X_T}{X_{Tm}} \right) X_T \right] - \beta \left[\mu_{cell} \left(1 - \frac{X_{cell}}{X_{cellm}} \right) X_T \right] X_{cell} + DC_i - DC \quad (6)$$

Where C is the cellulose concentration, α and β are kinetic constants. DC_i is the cellulose mass flow. In a bioreactor operated at batch or continuous mode, the cellulase concentration can be modeled considering the fresh medium feed rate (continuous) and the product formation rate, resulting in Eq. (7):

$$\frac{dP}{dt} = q_P X_{cell} - k_{dP} P - DC \quad (7)$$

k_{dP} cellulase deactivation and q_p is the product formation rate. The latter is calculated according to Eq. (8):

$$q_P = q_{Pmax} \left(1 - \frac{P}{Pm} \right) \left(\frac{1}{a \frac{C^2}{k_i} + 1} \right) \quad (8)$$

q_{Pmax} , Pm , a and k_i are model kinetic constants for cellulase kinetics. Table 1 shows the kinetic parameters used for all simulations. The latter was taken from previous experimental data [3]

Table 1: Kinetic parameter used for simulations

Parameter	Value
μ_{max} (h^{-1})	0.48
k_c (gL^{-1})	6.00
X_{Tm} (gL^{-1})	12.0
k_{dmax} (h^{-1})	0.095
$\mu_{cellmax}$ (h^{-1})	0.25
k_{di} (g/L^{-1})	0.44
k_{cell} (g/L^{-1})	2.84
X_{cellm} (gL^{-1})	5.76
k_{dcell} (h^{-1})	0.38
α (-)	0.069
β (-)	0.21
DC_i ($\text{gL}^{-1}\text{h}^{-1}$)	0.5
k_{dP} (h^{-1})	0.002
q_{Pmax} (h^{-1})	23.5
Pm (FPUL^{-1})	2513
a (-)	0.0

k_i (gL ⁻¹)	1.91
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The initial conditions are defined in Table 2.

Table 2. Initial conditions for cellulase production Feed-batch simulations

Parameter	Value	Units
X_{T0}	0.40	g/L
X_{cell}	0.00	g/L
C_0	10.0	g/L
P_0	0.00	FPU/L

The Runge-Kutta 45 numerical method was used with Matlab R2017b software to solve the differential equations proposed in this research.

3. Results and Discussions

The main objective of this research was the evaluation of two operating modes for the production of the cellulase enzyme from the *Trichoderma harzianum* fungus. The above was approached from the point of view of computational simulation. That is why the kinetic constants were taken from previous researches [3]. In such a way, batch and the continuous mode were fed with the parameters mentioned in Table 1.

To develop the mathematical model, the mass balance equations that govern a bioprocess in a dynamic state operated in batch and continuous mode were taken as a fundamental basis. Figure 1 shows the results of the simulation of the microbial growth of total cells (X), active cells (Xact), the cellulose consumption profile (Cc), and the enzymatic cellulase production (Cf) described by the mathematical modeling shown in this research.

The results obtained from the simulations developed were developed using a concentration of 10g / L of cellulose and 0.4g / L of cells as initial conditions. The maximum cell growth values for the discontinuous feeding model identified in Figure 1 were 6 g / L in a time of 24.5 h. Interestingly, it was evidenced that, of the mentioned concentration of total cells, only 1.8 g / L of cells manage to be active for the enzymatic production of cellulases.

The above can be argued considering the different metabolic states that a microorganism can develop when cultured in a transient state [10-12]. The synthesis of new cells can be negatively affected by the substrate concentration gradients, viability, and microenvironments generated in a bioreactor. All these phenomena are captured by the cellular deactivation constant k_{dcell} exposed in the mathematical model shown in this investigation.

Concerning the consumption of the substrate, a total conversion is observed approximately after 40 hours of the process. At this point in the fermentation, the *Trichoderma* fungus reaches its maximum level of cellulase production so that a simulated value of 800 FPU / L is obtained when the bioreactor is operated in batch mode.

According to the cellulase simulation using batch mode, an enzyme yield of up to around 80 FPU / gL could be obtained if the process is started with a substrate load of 10 g / L and the size of inoculum of the fungus centered at a value close to 0.4 g / L.

However, the results shown in Figure 1 suggest microbial growth is drastically affected at levels below 4 g / L of the substrate. For example, above, considering the results of Figure 1 (b), the concentration of active cells is spontaneously reduced from the mentioned value of cellulose.

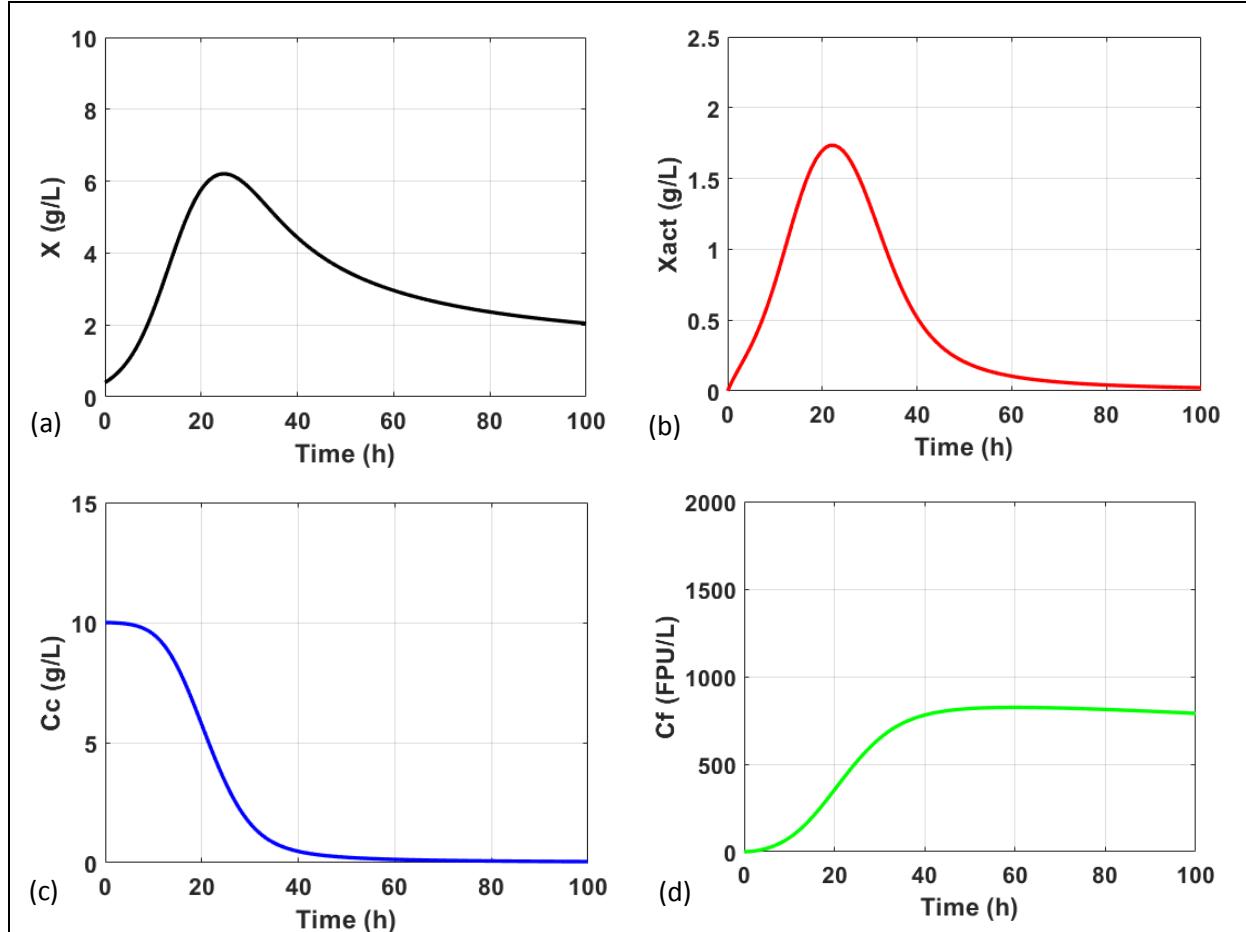


Figure 1. Cellulase production from a batch operating mode. (a) Total Biomass, (b) Active cells, (c) Cellose uptake, and (d) Cellulase concentration

Based on the findings found in the batch mode simulation for the production of cellulases, the operation of the bioreactor in continuous mode was proposed in such a way that the cellulose concentration could remain above 4 g / L and verify if significant changes arise in the concentration of active cells and the production of cellulases. The results of this second strategy are shown in Figure 2.

According to the results obtained in Figure 2, a cellular production of 8 g / L and a maximum cellulase level of 1800 PUF / L is evidenced. Interestingly, cellulase levels managed to stay above 4 g / L as initially planned based on the information previously exposed, in such a way that the mathematical model responds satisfactorily to the mentioned stimulus.

The latter is considered a starting point for further cellulase improvements focused on maximizing the bioprocess yield in a bioreactor operated in continuous mode. Contrary to batch mode, better cellulase production is observed in Figure 3 using the continuous strategy, with a maximal value of 1800 FPU/L of cellulase. The latter means an increase of more than twice the value found using the batch methodology.

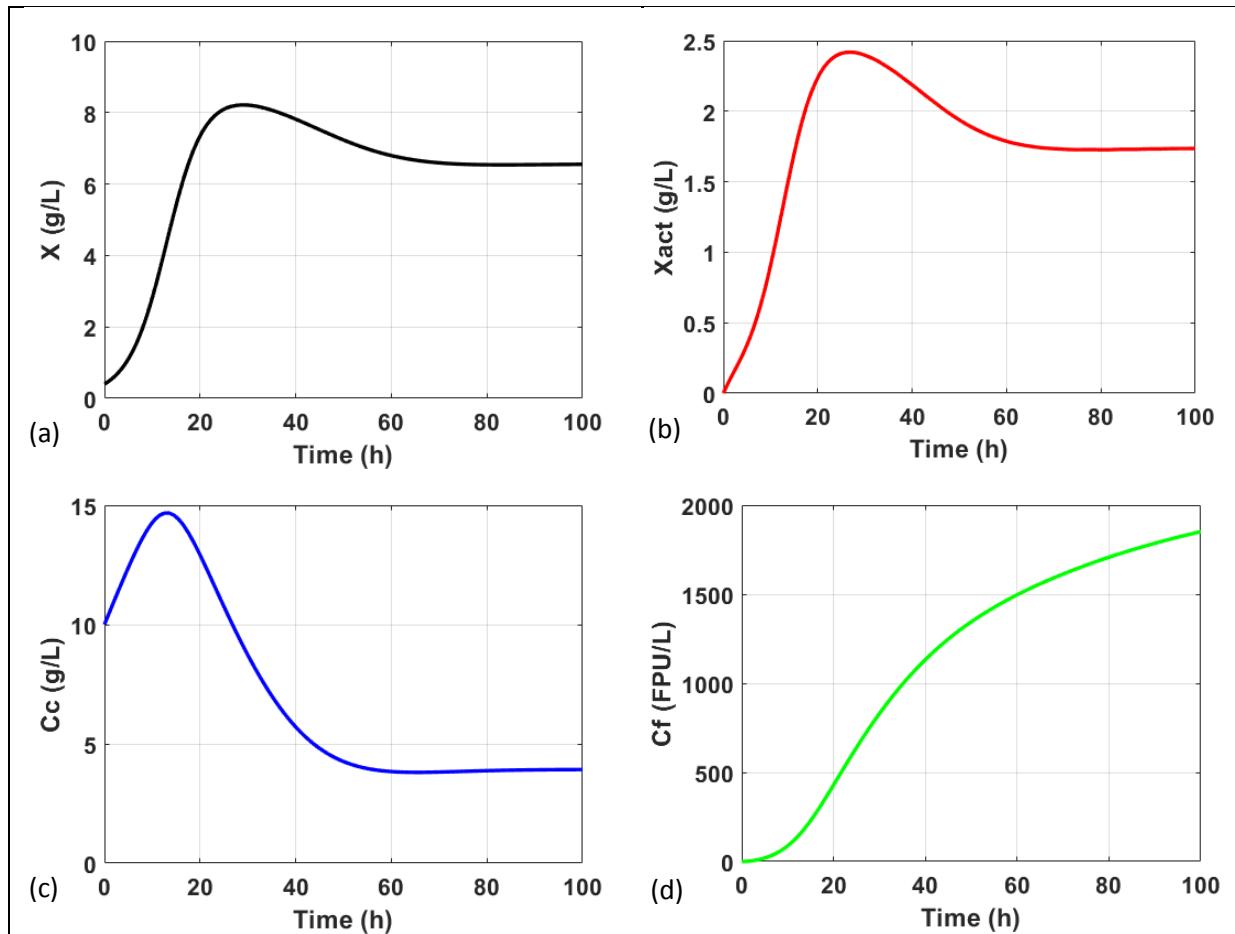


Figure 2. Cellulase production from a continuous operating mode. (a) Total Biomass, (b)Active cells, (c) Cellose uptake, and (d) Cellulase concentration

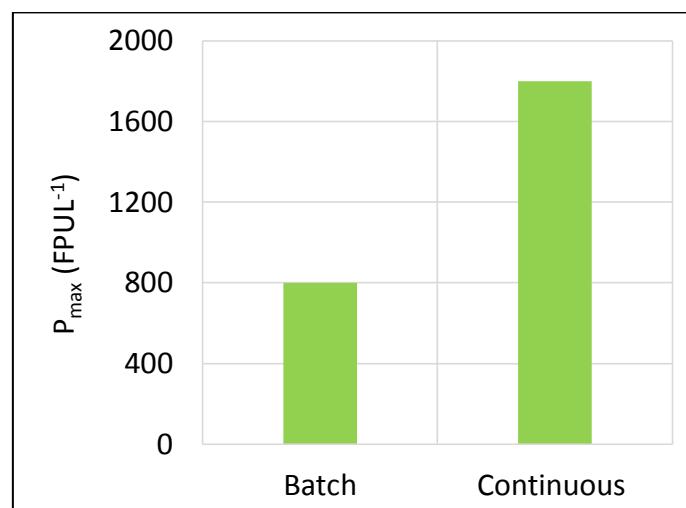


Figure 3. Maximal cellulase concentration reached for batch and continuous operating mode

Unlike the continuous mode, it is defined as a highly efficient process in consuming the inducing substrate in a low time, thus developing highly reliable productivity. When carrying out the detailed operating mode comparison, it is determined that the continuous mode is the most suitable for any biotechnological process [13-14]. Since it has greater indefinite productivity to acquire a product of interest, the latter implies a process control in a steady-state and handles relatively short cultivation times that guarantee better performance for activities in different areas such as pharmaceutical, industrial, medical, and food, and biotechnology. Thus, the mathematical model developed in this work can be adapted for other microorganisms and inducing substrates (such as sugarcane bagasse) to develop new strategies to continue increasing cellulase productivity until reaching the desired value.

4. Conclusions

Enzyme production is a topic of interest in biotechnology productivity enhancement. Therefore, simulating cellulase production from *Trichoderma harzianum* was evaluated in this research. Thus, the handling of batch and continuous modes of operation is induced, where noticeable efficiency is obtained in the continuous bioreactor, reaching around 1800 FPU/L of cellulase, meaning a more significant concentration than those obtained using a batch mode (800 FUP/L). Likewise, the effectiveness of the continuous mode is evidenced and compared with references, demonstrating its quality, productivity, and performance, motivating the optimization and improvements on an industrial scale.

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