# Operating Mode Effect on Lipids Production from *Rhodotorula mucilaginosa:* Modelling and Simulation Trends



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**Abstract.** A sustainable alternative for fossil fuels substitution is the production of biodiesel from natural lipids. Mainly, such oil used is in the form of triglycerides and is characterized by being potentially renewable, non-toxic, and biodegradable, thus making it a sustainable product. For producing such biofuel, yeast oil is one of the most viable and currently known options, obtaining theoretically optimal results. In this article, kinetic parameters from the oleaginous yeast *Rhodotorula mucilaginosa* will simulate lipid accumulation capacity based on different bioreactor operating modes. Likewise, The Matlab software is used to simulate batch and continuous operating modes to establish comparisons between the productions obtained and their respective yields. The results obtained for the batch mode were: 14 g/L for the growth of biomass and an estimate of 0.22 g/g for lipid production. Likewise, for the continuous mode, its results were: 19 g/L for biomass growth and a value of 0.30 g/g in lipid production. The simulated results in continuous mode would demonstrate the effectiveness of its implementation since a more significant lipid accumulation is reached.

## 1. Introduction

Currently, biofuels production has been characterized as a central topic of interest in bioprocess technology. Its importance is focused on producing energy from biomass sources, thus becoming a promising alternative to petroleum derivatives. In this context, a sustainable option is producing biodiesel in industry obtained from natural lipids such as vegetable oils and/or animal fats to replace fossil fuels [1]. Commonly, the oil is used as triglycerides (glycerol molecule linked to three fatty acid molecules) which are comparable to conventional vegetable oils [2], having the advantage that they are potentially renewable, non-toxic, and biodegradable sources, resulting in a sustainable product [3].

In the case of biodiesel, the primary microbial producer is microalgae. However, an alternative is the oil-producing yeasts with yields similar to those reported theoretically. Therefore, the *Rhodotorula mucilaginosa* species is distinguished by a glow that surrounds the colonies, which is primarily caused by its high lipid accumulation capacity [4], being classified as oleaginous microorganisms since they are capable of producing and accumulating at least 20% of oil concerning the weight of its dry biomass.

According to studies, it is affirmed that it can have a dry weight made up of 70% of lipids, of which 85% are fatty acids [5]. The species *R. mucilaginosa* belongs to the *Sporidiobolales* order of the Microbotryomycetes and has a facultative aerobic respiratory metabolism. That is, it can grow in environments with a high amount of oxygen or in those with low oxygen availability, such as rivers, lakes, and waters residual oil deposits, among others [4].

Thus, lipid synthesis benefit or performance is closely related to the presence of nutrients in the culture medium due to the attention given by the microorganism to nutritional demand. Therefore, a relevant aspect is that when there is a shortage of a macronutrient, usually nitrogen, the carbon absorption in excess is used to produce the fat. For this reason, the lipid accumulation is given by the C/N ratio [2]. Among the main microorganisms, yeasts have advantages in oil production since they have a high yield when using glycerol as a carbon source, which also benefits biodiesel productivity [6].

The biodiesel production depends on the bioprocess operating mode in which biomass and substrate are added, and a product of interest is obtained, in the present case, microbial lipids. The Batch (discontinuous) operating mode consists of a storage tank with an agitator and a heat exchanger for its standard platform since it is a device designed to transfer heat between 2 fluids or between a fluid and a solid [7]. These operating bioreactors are characterized because there are no inputs or outputs of components. The materials are simply introduced before starting the process in the tank, and the reaction occurs during a respective time. However, it is generally required to have a temperature, pressure, and volume controls [8].

On the other hand, the continuous mode has seen a resurgence of interest related to its potential to challenge the established batch production mode developing and evaluating continuous technologies for potential benefits [9].

Consequently, the continuous operating model consists of a tank made up of a feed pump where the fresh culture medium is added and a discharge pump that sucks inside the reactor [10]. Generating inhibitory agents and toxins are counteracted by suction of the products. Continuous mode is used in different scenarios when it is desirable to have continuous evolution of a mixed population of cells and in wastewater treatment or, at best, lipid production [7].

Considering the factors mentioned, this article aims to evaluate the Matlab software to simulate and determine the optimal operating mode for lipids accumulation from *R. mucilaginous*. Consequently, two operating modes are proposed batch and continuous to establish the respective comparisons through simulations and specify the main parameters to verify, such as extreme performance and industrial-scale production.

## 2. Methodology

The batch reactor or discontinuous reactor consists of introducing the components involved before the process. Therefore, the entry and exit rates remain null. In the same way, the products are removed at the end of the process. Kinetic parameters are taken from previous studies [11].

The fundamental equation of the reactor involves accumulation and inlet and outlet flow rates is shown in Eq. (1):

$$\frac{dM}{dt} = M_{in} - M_{out} + R_g - R_c \tag{1}$$

In which, $M_{in}$ and $M_{out}$ are mass added and extracted from the bioreactor, with a value of 0.0 for the batch operating mode. $R_g$ is the rate of species generation and $R_c$ is species uptake rate. The biomass growth of *R. mucilaginous* was determined from Eq. (2), in which $X$ is the cell concentration.

$$\frac{dX}{dt} = \mu X \tag{2}$$

Where μ is the specific growth rate, which relates the cell formation rate to the substrate concentration consumed in the bioreactor and is calculated in Eq.(3):

$$\mu = \frac{\mu_{max} S}{k_s + S} \tag{3}$$

Where $\mu_{max}$ is the maximum microbial growth rate and $k_s$ is a saturation constant. According to the referenced experimental data [11], glucose is used as a limiting substrate. That is why glucose impacts in batch mode are simulated using Eq. (4):

$$\frac{dS}{dt} = -\left(\frac{\mu}{Y_{x/s}} + m_s\right) X \tag{4}$$

Where $Yxs$ is the yield of *R. mucilaginous* cells produced per unit of glucose consumed, lipids production in a bioreactor operated in batch mode can be modeled using the Eq. (5):

$$\frac{dP}{dt} = Y_{P/s}\left(\frac{\mu}{Y_{x/s}} + m_s\right) X \tag{5}$$

Where $P$ is the Lipid concentration that occurs as a function of time. $\alpha$ and $\beta$ are kinetic parameters. Table 1 shows the kinetic parameters used for all simulations.

**Table 1:** Kinetic parameter used for simulations

| Parameter | Value |
|---|---|
| $\mu_{max}$ ($h^{-1}$) | 0.10 |
| $k_s$ ($gL^{-1}$) | 2.00 |
| $Yxs$ ($gg^{-1}$) | 0.50 |
| $Y_{P/s}$ ($gg^{-1}$) | 0.008 |
| $m_s$ ($gg^{-1}h^{-1}$) | $3.5*10^{-5}$ |

The initial conditions and parameters for the simulation of lipid production are presented in Table 2.

**Table 2:** Initial Conditions and parameters used for simulations

| Parameter | Value |
|---|---|
| $X_0$ ($gL^{-1}$) | 0.25 |
| $S_0$ ($gL^{-1}$) | 28.0 |
| $P_0$ ($gL^{-1}$) | 0.00 |

The fundamental equation in a reactor compromises the mass in and oud mentioned before in eq. (1). So that, biomass equation in continuous mode is modeled as shown in Eq. 6.

$$\frac{dX}{dt} = \mu X - \frac{F}{V} X \tag{6}$$

Thus, the volume $V$ in a continuous reactor remains constant during the process. Following this equation, we have the dynamic equation of the biomass concentration X, considering that the input is zero because the biomass enters the reactor sterile.

Also, including the mass in and out for substrate modeling, the Eq. (7) results in:

$$\frac{dS}{dt} = \frac{F}{V}S_i - \frac{F}{V}S - \left(\frac{\mu}{Y_{x/s}} + m_s\right)X \quad (7)$$

Where $S_i$ is the glucose concentration fed with the stream $F$. Finally, the impact of continuous operating mode on product concentration is modeled in Eq. (8) as:

$$\frac{dP}{dt} = Y_{P/s}\left(\frac{\mu}{Y_{x/s}} + m_s\right)X - \frac{F}{V}P \quad (8)$$

The initial conditions are shown in Table 2. In this research, V and F are defined as 3 L and 0.1 L/h, respectively.

**3. Results and Discussions**

Considering that it is required to recall the primary purpose of this research article, comparisons between two operating modes are performed for analyzing lipids production from *R. mucilaginous*. A mathematical modeling framework is presented for simulating each operating modes (batch and continuous) based on the latter. Performance and quality on an industrial scale are considered for its production from constitutive equations solved numerically using the Matlab software.

The modes presented in this study incorporates control theory tools to determine lipid production so that, knowing the appropriate protocols, criteria, and operating mode, the processes can be optimized. Next, the different data obtained in the two operating modes previously presented will be disclosed. Figure 1 the biomass growth, substrate consumption, and lipids accumulation are shown.

It is important to note that the following initial conditions were used: 0.25 g/L of biomass, 28 g/L of glucose. Regarding the batch mode, a biomass growth of approximately 14 g/L was obtained. Likewise, the rate of glucose expenditure is reflected, where it can be seen that the total consumption of substrate was carried out in an estimated time of 45 h.

Now, analyzing the data obtained from the product, it is possible to observe the lipids in the biomass with an estimate of 0.22 g/g. According to the continuous mode shown in Figure 2, the biomass growth obtained was 19 g/L. In turn, the glucose consumption occurred closely in 75 h, thus resulting in a value of 0.30 g/g in lipid production.

Previous studies carried out with *Rhodotorula mucilaginosa* focused on lipids production using the batch, and continuous modes are limited. However, few studies have reported carbon sources as a substrate for lipids' production [12]. Based on the above, this paper's motivation is to present a framework modeling considering comparing both batch and continuous operating modes to optimize lipid accumulation.

Previous reports [12] based on the genetically modified *Yarrowia lipolytica* suggest a similar lipid accumulation using molasses and glycerol as carbon sources in a batch bioreactor. However, it is shown that *Rhodotorula mucilaginosa* is characterized by more advantages. The latter because of its capacity to produce carotenoids and enzymes such as L-phenylalanine, ammonia-lyase, and D-ammonia acid oxidase, compounds of high industrial relevance [13].

According to a study [14-15], it was demonstrated experimentally that the continuous mode has advantages over batch processes. Therefore, making an analogy concerning the above and considering the results simulated in the present study, it can be inferred that lipid production would be more feasible than the batch operating mode since lipid concentration reached a value closed to 0.33 g/g mentioned before. Furthermore, the continuous bioprocessing mode also allows high cell densities, which are crucial for producing microbial oil.

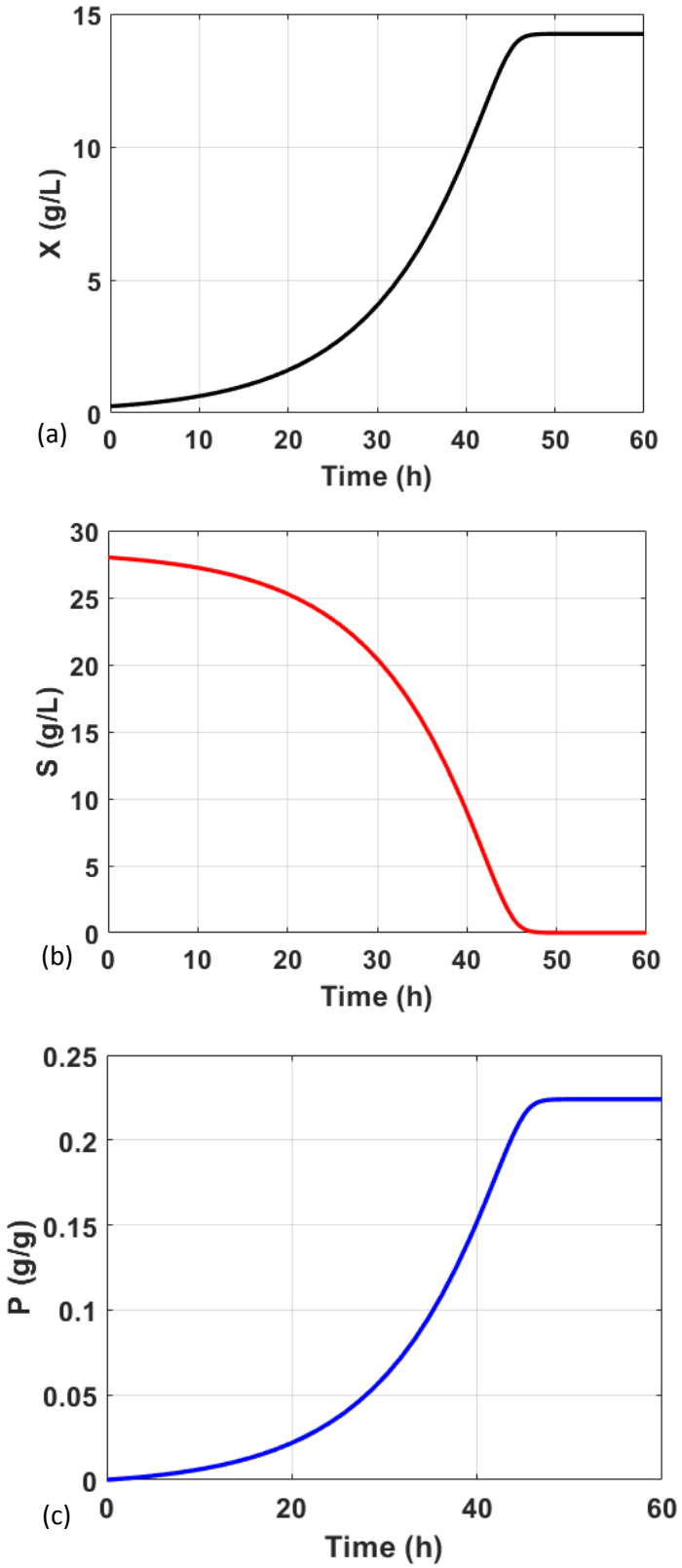


**Figure 1.** Lipids production simulated at batch operating mode (a) Biomass, (b) Glucose, and (c) Product.

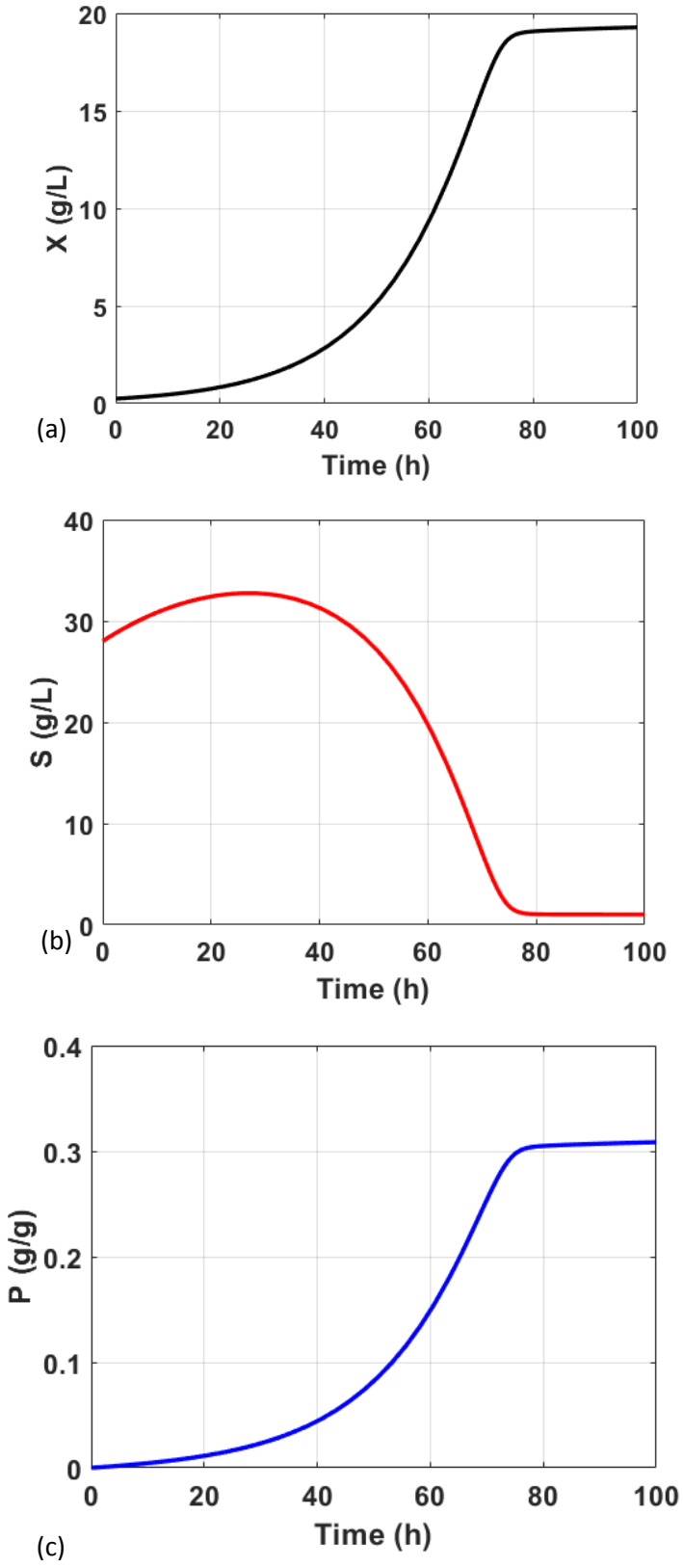


**Figure 2.** Lipids production simulated at continuous operating mode (a) Biomass, (b) Glucose, and (c) Product.

According to Figure 3, it is possible to detail the efficiency in productivity concerning the continuous mode compared to the batch mode since biomass and lipid productivity resulted in 0.61 g/L.h and 0.01 g/g.h, respectively continuous bioprocessing. The latter confirms that the continuous mode reveals superior behaviors in opposition to the batch mode regarding productivity, utility, and quality on an industrial scale.

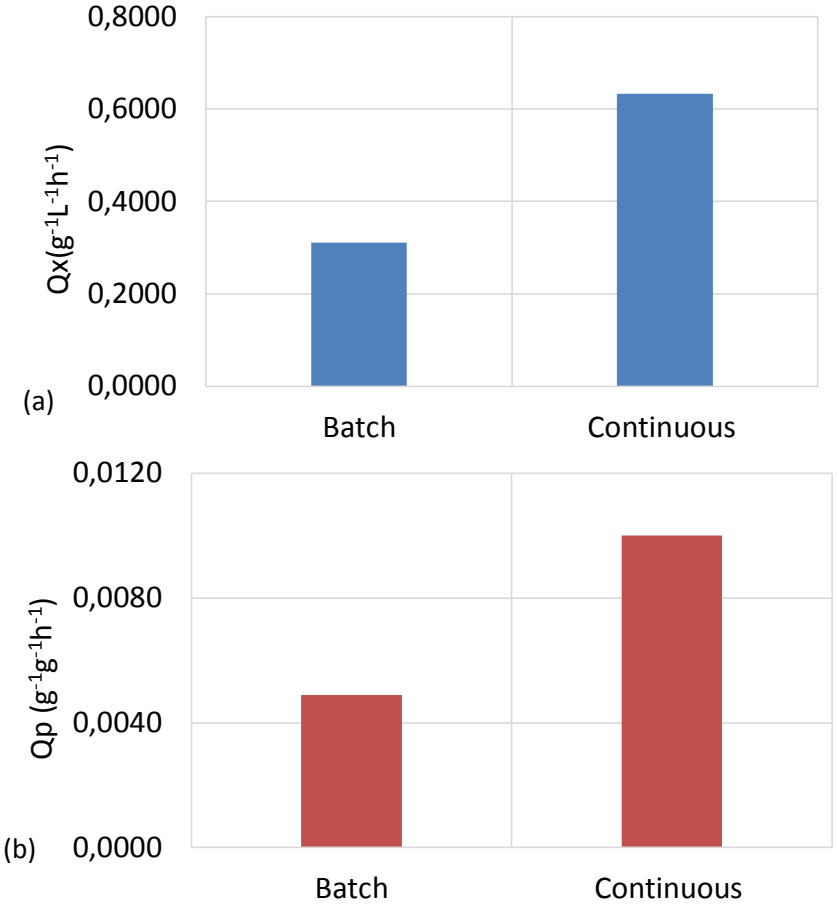


**Figure 3.** Biomass (a) and Lipid (b) productivities for both batch and continuous operating mode.

## 4. Conclusions

Biofuels are a topic of interest in the oil productivity enhancement from oleaginous yeasts. Therefore, simulating of oil production from *Rhodotorula mucilaginosa* 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 0.30 g/g of oil, meaning a more significant accumulation of lipids. 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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