Modelling of DHA Production from *S. Limacinum* ouc88: fed-batch perspectives

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Abstract. Biotechnology and its need to improve industrial processes have shown the need for techniques that allow processes optimization. Computer simulation offers the advantage of determining production prospects without significant resources and experimentation time. That is why a kinetic study of DHA (docosahexaenoic acid) production from *Schizochytrium limacinum* OUC88 was developed in this investigation. Based on the above, a mathematical approach to simulate DHA production in a fed-batch model is proposed in this work, using Matlab software. The experimental data for determining kinetic parameters were taken from previous investigations, and a simulated DHA level in the fed-batch mode of 150 g/L was reached. On the contrary, the simulated results in batch mode present only a maximum value of 30 g/L, demonstrating the effectiveness of the fed-batch implementation with perspectives on improving processes.

1. Introduction

Docosahexaenoic acid (DHA) is a metabolite commonly found in vertebrates and invertebrates [1] and with multiple health benefits. A significant concentration of DHA has been required in the brain to develop the retina and nervous system [2] properly. DHA belongs to polyunsaturated fatty acids (PUFAs), and the primary source for a regular diet can be found in fish oil. However, its composition is highly variable, in such a way that it depends on various factors such as the fish species and the extraction techniques. Based on the above, new DHA production techniques are currently being implemented. Some microorganisms can synthesize DHA through fermentative processes such as *Schizochytrium limacinum* SR21 [3], *Schizochytrium sp*. S31 [4], 2003; [4], *Schizochytrium mangrovei* [5] and *S. limacinum* OUC88 [6].

The constant concern of the industrial sector for the improvement of biotechnological processes has shown the need for techniques that allow processes optimization [7-10]. Obtaining DHA by biotechnology from microorganisms offers considerable advantages compared to traditional obtaining from fish oil. The preceding, taking into account that the environment is controlled and production can be manipulated according to the requirements and the equipment operation mode. The modes traditionally used in bioprocesses being batch, fed-batch, and continuous. A batch process operates in a closed system [11-12]. All matter is added to the system at the beginning of the process, the process is closed, and the products are collected only when the process is complete. A semi-continuous process allows mass input or output, but not both. A continuous process allows the entry and exit of matter. If the entry and exit speeds are equal, the continuous process can operate indefinitely.

The fed-batch technique is an operation in which the nutrients necessary for cell growth or product formation (DHA) are fed to the reactor gradually. The harvest of the products of a fed-batch process is done at the end of the process.

The carbon, nitrogen, phosphates, and other nutrients are intermittently added to the bioreactor or continue to manipulate the feed flows during the process to use the culture by fed-batch takes advantage of the fact that the concentration of the limiting substrate is maintained in low quantities during fermentation thus avoiding the repressive and toxic effects of a high concentration of the substrate. Furthermore, the systems by fed-batch allow for manipulating the microorganism growth rates. The last thing is associated with physiological properties that allow improving the produced substances' selectivity. A fed-batch operation offers methods for regulating the metabolite concentration that affects the reaction by controlling feed rates. The result generates a productive advantage compared to a batch strategy or continuous fermentation.

Based on the above, the mathematical modeling of production in fed-batch mode is considered a critical stage for identifying the best trends that allow elucidating the improvement of DHA production at the bioreactor level. In such a way, it is possible to know the DHA production kinetics in a bioreactor operated in a fed-batch mode without the need to make a significant investment in resources and experimentation time using transient mass balances. That is why, in this research, a kinetic study was carried out by simulating the DHA (docosahexaenoic acid) production from *Schizochytrium limacinum* OUC88.

2. Methodology

In this research, previous experimental data [13] were used to determine the kinetic parameters of the proposed model, which allows simulating DHA production in fed-batch mode, from the microorganism *Schizochytrium limacinum* OUC88.

The culture volume contained in the bioreactor depends on inflow and outflow components in such a way that volume can be modeled according to Eq. (1):

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

In which, F is the fresh culture medium feed flow and F_{out} are the programmed discharges of the depleted medium. The biomass growth of *S. limacinum* was determined from Eq (1), in which C_x is the cell concentration and V is the volume of the bioreactor.

$$\frac{dC_xV}{dt} = \mu C_xV - F_{out}C_x \tag{2}$$

Where μ is the specific growth rate, which relates the cell formation rate to the substrate concentration consumed in the bioreactor and is calculated as:

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

Where μ_{max} is the maximum microbial growth rate and k_s is a saturation constant. According to the referenced experimental data [13], glucose is used as a limiting substrate. That is why glucose impacts in fed-batch mode are simulated using Eq. (4):

$$\frac{dC_sV}{dt} = FC_{si} - q_sC_xV - F_{out}C_s \tag{4}$$

The term C_{si} refers to the glucose concentration fed in fed-batch mode and q_s is the specific glucose uptake rate and is calculated as:

$$q_s = \frac{\mu}{Yxs} \quad (5)$$

Where Yxs is the yield of *S. limacinum* cells produced per unit of glucose consumed. DHA production in a bioreactor operated in fed-batch mode can be controlled by manipulating the feed rate of fresh medium and the rate of extraction of spent medium, in such a way that:

$$\frac{dC_P V}{dt} = q_P C_x V - F_{out} C_P \quad (6)$$

Where C_P is the DHA concentration that occurs as a function of time and q_P is the specific rate of DHA formation. In this research, q_P is calculated with the Luedeking-Piret equation following previous findings [13]:

$$q_P = k_1 + k_2 \mu \quad (7)$$

This model was initially proposed for the formation of lactic acid from *Lactobacillus delbrucckii*. The mentioned model indirectly relates DHA production to microbial growth [14].

The simulations were carried out with the MATLAB R2017b software. The Runge-Kutta 45 method was used to numerically solve the proposed mathematical model to simulate DHA production from *S. limacinum*.

Table 1 shows the kinetic constants used to carry out the simulations in Fed-batch mode. These were determined from experimental data reported in previous research.

Table 1: Kinetic parameter used for simulations

Parameter	Value
μ_{max} (h^{-1})	0.080
k_s (gL^{-1})	0.100
Yxs (gg^{-1})	0.460
k_1 (gg^{-1})	0.139
k_2 ($gg^{-1}h^{-1}$)	0.001

The initial conditions and parameters for the simulation of DHA production in Fed-Batch mode are presented in Table 2. The process starts in batch mode during the first 60 hours. After this time, a feed flow rate F is activated at a rate of 1 L/h.

When the 120 hours of fermentation are reached, the feed flow increases to a constant value of 2 L/h for 30 hours. Then the feed was increased to 20 L/h until the end of the process. The discharge was programmed at 119 hours with a rate of 20 L/h during an hour

Table 2: Initial Conditions and parameters used for simulations

Parameter	Value
C_{x0} (gL^{-1})	0.370
C_{s0} (gL^{-1})	59.37
C_{P0} (gL^{-1})	0.000
V_0 (L)	0.100

3. Results and Discussions

The purpose of this research was to identify and describe improvements in DHA production by *S. Limacinum* ouc88 simulating the fed-batch operating mode using the software Matlab. Figure 1 shows that, initially, the maximum cell concentration was 27.68 g/L at 54.5 hours. After the first substrate feeding, the maximum cell concentration reached 23.0 g/L at 115.66 hours.

After the first and second substrate feeding, the cell concentration reached 27.28 g/L at 160 hours and remained constant after the last feeding. As shown in Figure 1, the biomass growth does not increase after the last feeding but remains constant over time. The latter is because of the biomass cell dilution due to each feeding.

It should be noted that the initial concentration of substrate and fed substrate was 59.37 g/L. DHA is an essential structural component of membrane phospholipids. Also, it is the substrate for the formation of a series of lipid derivatives called eicosanoids (derivatives of 20 carbon atoms in the case of AA and EPA) and docosanoids (derivatives of 22 carbon atoms) [2].

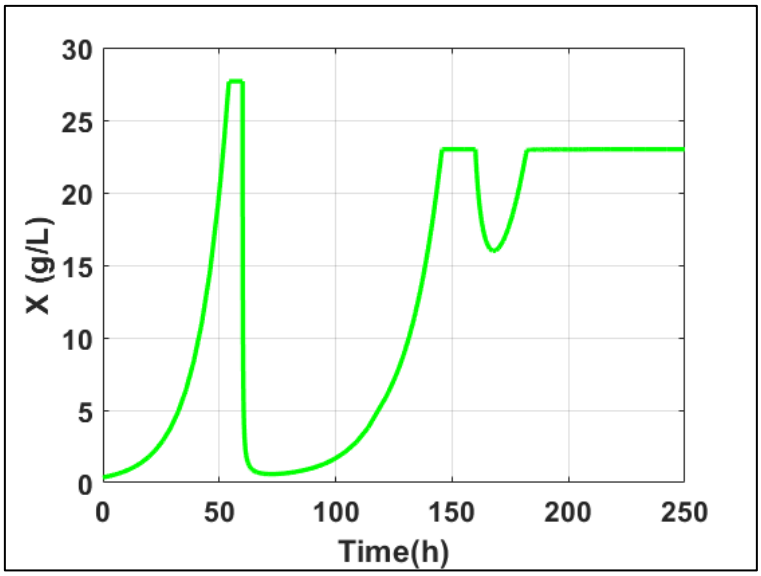

Figure 1. Simulation results for Biomass (g/L) using the fed-batch strategy.

The evolution of the substrate during the process is shown in Figure 2 where it is consumed more quickly in each feeding, each with a concentration of 59.37 g/L due to the increase in total cells speed of substrate consumption.

According to Figure 2, feeding the glucose at 20 L/h for 160 hours would lead a substrate depletion since it tends to 0.0 g/L. Therefore, different strategies should be improved.

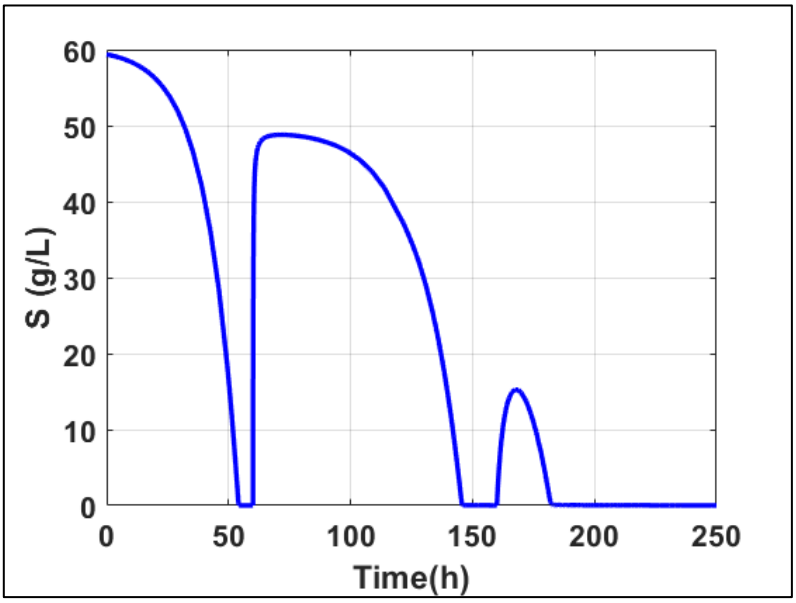

Figure 2. Simulation results for substrate (g/L) using the fed-batch strategy.

In Figure 2, it can be seen how the substrate keeps in a short latency state at first hours, and the exponential growth begins while substrate decreases drastically up to the first 60 hours. Based on the mathematical framework DHA was associated with the rate of cell growth. Figure 4 shows the DHA production using the fed-batch strategy. It is shown that DHA is increased from 70 to 150 g/L using the combined fed-batch strategy proposed in this research. However, after the first pulse has been added, a decrease from 60 hours is observed in the simulations. This is because the fed flow causes a critical dilution resulting in a lower DHA concentration. Then, As the cells continue growing, DHA starts to accumulate to finally reach 150 g/L, as shown in Fig 3.

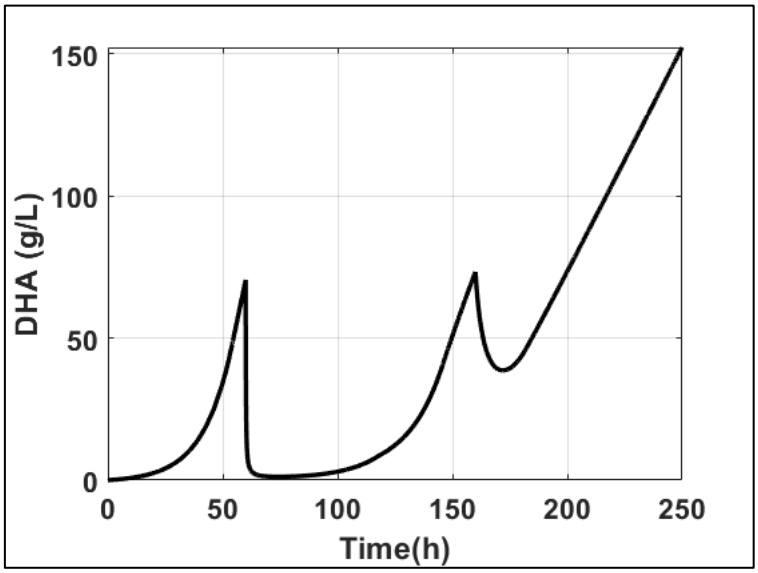

Figure 3. Simulation results for Feed-batch tannase production. (a) Biomass, (c) Substrate, and (c) Product.

Based on Eq. (1), the working volume is affected by the feed rate. Hence, the filling rate is variable during fermentation in Fed-batch mode. Considering the results obtained in this research (Figure 4), a volume of almost 140 liters is reached at 159 hours of operation at the end of the second feeding pulse of 2 L/h. Once the fermentation is finished in Fed-batch mode at 250 hours, the filling size is closed to 3000 liters. According to the above, the design size of the bioreactor requires a size greater than 3000 liters.

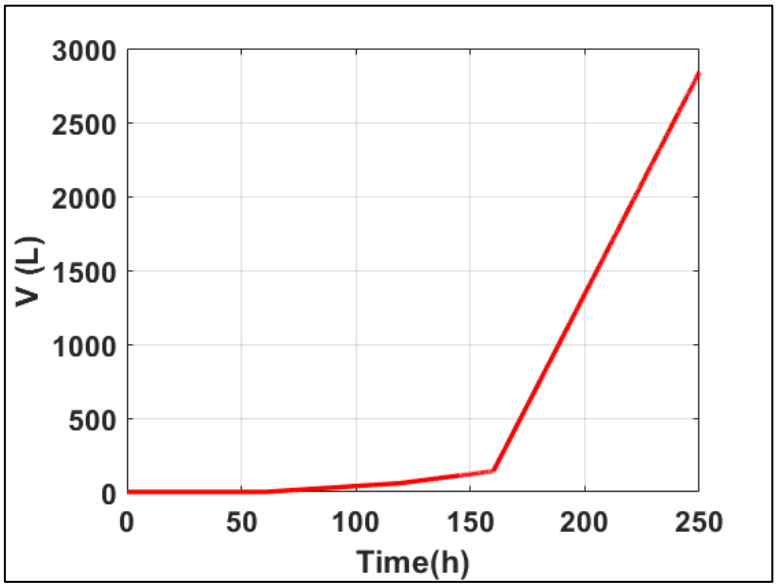

Figure 4. Bioreactor volume profile simulated for Feed-batch DHA production.

4. Conclusions

This research proposed an operation strategy in Fed-batch mode with programmed substrate feeding stages to improve DHA production. The Fed-batch mode proposed controls the DHA concentrations. The above is constituted as a starting point for future optimization research. The results obtained in this research may be promising for the biotechnology industry. It is possible to identify improvements to an industrial process through computer-aided design techniques without performing excessive experimentation.

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