

## MATHEMATICAL MODELING OF FILAMENTOUS FUNGAL SINGLE CELL OIL PRODUCTION IN SUGARCANE-BASED CULTURE MEDIA

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### ABSTRACT

Microbial lipids (or Single Cell Oil) have been highlighted as a promising alternative source for biodiesel production and nutraceutical supplementation. In this study, cultivations of *Mucor circinelloides* for lipid production were analyzed, utilizing mathematical modeling as a tool to investigate process kinetics. The lipid production processes were conducted using sugarcane molasses in basal medium (SM-BM), basal medium supplemented with corn steep liquor (SM-BM-CSL), and basal medium supplemented with synthetic nutrients (SM-BM-SN). The results indicated that the model fitting using a genetic algorithm with 10 generations and optimization via the Runge-Kutta method was highly satisfactory. The kinetic parameters obtained demonstrate that the SM-BM-SN medium yields superior lipid production results.

**Keywords:** kinetics, lipids production, microbial lipids, *Mucor circinelloides*.

### 1 INTRODUCTION

Single Cell Oil is a promising source of lipids to be used in many biorefinery context applications. *Mucor circinelloides* has been highlighted as an outstanding microorganism for Single Cell Oil production using a wide array of substrates, including agro-industrial by-products as sugarcane molasses. Regarding its applications, as biodiesel raw material it does not compete with the food sector and presents good properties and productivity. Also, in food and pharmaceutical industries it shows potential to be used due to their nutraceutical properties achieved by its composition of unsaturated fatty acids and carotenoids content<sup>1</sup>.

In the realm of biotechnological lipid production, the application of modeling techniques proves to be paramount. These techniques furnish critical kinetic parameters that are essential not only for the precise monitoring of the production process but also for a comprehensive understanding of the underlying biochemical mechanisms. By integrating modeling approaches, researchers can gain deeper insights into the dynamic interactions and regulatory pathways that govern lipid biosynthesis. This enhanced understanding facilitates the optimization of production conditions, ultimately leading to more efficient and scalable biotechnological processes. Consequently, the role of modeling in this context is indispensable for advancing both theoretical knowledge and practical applications in the field of lipid biotechnology<sup>1,2</sup>. In the present work, modeling of the lipid production process was carried out by *Mucor circinelloides* with different cultivation media in batch mode.

### 2 MATERIAL & METHODS

Cultivations were performed using sugarcane molasses as carbon source (40 g L<sup>-1</sup> total sugar) in submerged cultivation of *Mucor circinelloides* (URM 4182) using a 1 L bioreactor (Bioflo 115, Eppendorf) containing 0.7 L as work volume. Bioreactor parameters were set as indicated by Bento et al. (2019)<sup>3</sup>. Influence of supplementation by Corn Steep Liquor (CSL) and semi-synthetic nutrients (SN) (ammonium sulfate, 1.5 g L<sup>-1</sup>, glutamic acid, 1.5 g L<sup>-1</sup>, nicotinic acid, 1 mg L<sup>-1</sup>, thiamine, 1 mg L<sup>-1</sup> and yeast extract, 0.5 g L<sup>-1</sup>) were evaluated in biomass growth, lipid production and substrate consumption. Lipids were extracted using ethanol in a microwave-assisted method as described by Bento et al. (2020)<sup>1</sup>.

These processes were modeled from the mass balance of cells (X), substrate (S) and lipids (P) resulting in the following system of ordinary differential equations (1-3). The modeling was based on the model proposed by Aggelis et al. (1995)<sup>4</sup> which used coefficients  $k_1$  and  $k_2$ .

$$\frac{dC_S}{dt} = -k_2 \cdot C_S \quad (1)$$

$$\frac{dC_X}{dt} = -\frac{dC_S}{dt} \cdot k_1 \cdot C_X = k_2 \cdot C_S \cdot k_1 \cdot C_X \quad (2)$$

$$\frac{dC_P}{dt} = \frac{dC_X}{dt} \cdot \frac{1}{Y_{X/P}} = \frac{(k_2 \cdot C_S \cdot k_1 \cdot C_X)}{Y_{X/P}} \quad (3)$$

where:  $C_S$  is the substrate concentration ( $\text{g L}^{-1}$ ),  $k_2$  is the substrate consumption constant ( $\text{h}^{-1}$ ),  $C_X$  is the cell concentration on a dry basis ( $\text{g L}^{-1}$ ),  $k_1$  is the related constant to the yield of substrate to cells ( $\text{L g}^{-1}$ ),  $C_P$  is the concentration of lipids ( $\text{g L}^{-1}$ ),  $Y_{X/P}$  is the coefficient that relates cell formation to lipid production ( $\text{g}_X \text{g}_P^{-1}$ ).

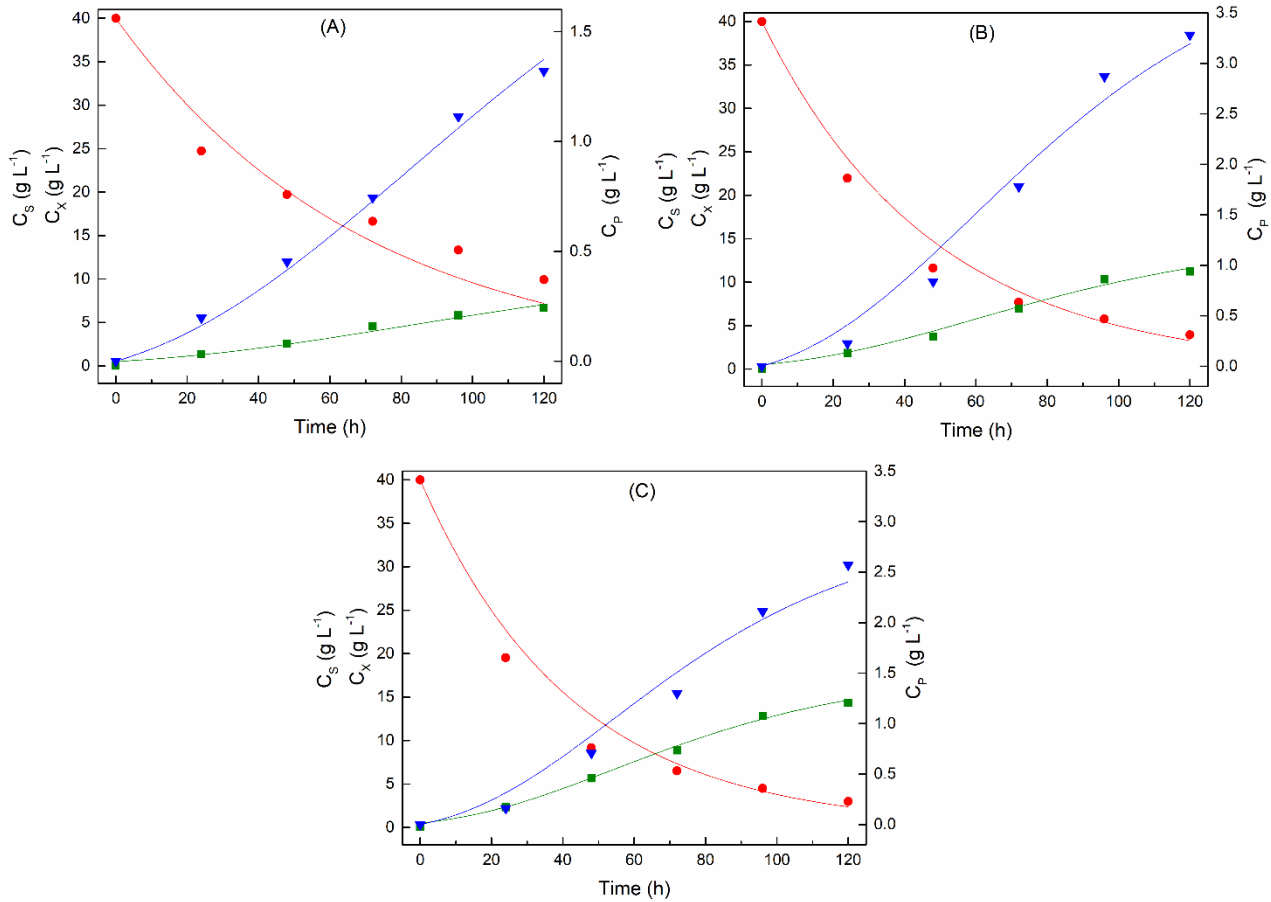
The model was adjusted to the experimental data of  $C_X$ ,  $C_S$  and  $C_P$ , estimating the values of the constants  $k_1$  and  $k_2$ . The model parameters were estimated using a genetic algorithm with 10 generations, a population of 100 individuals, a crossing probability of 70% and a mutation probability of 10%. The genetic algorithm was used together with the Runge-Kutta algorithm for the numerical solution of the differential equations. The criterion used to adjust the model was the minimization of the residual sum of squares (RSS) of the three modeled state variables ( $C_X$ ,  $C_S$  and  $C_P$ ), given by equation 4.

$$RSS = \sum_{i=1}^n (C_X - \hat{C}_X)^2 + \sum_{i=1}^n (C_S - \hat{C}_S)^2 + \sum_{i=1}^n (C_P - \hat{C}_P)^2 \quad (4)$$

where:  $n$  is the number of experimental points,  $C$  and is the experimental concentration for cells, substrate, and lipids, and  $\hat{C}$  is the concentration predicted by the model.

### 3 RESULTS & DISCUSSION

Figure 1 shows the mathematical model adjusted to experimental data from cultures with basal medium (SM-BM), basal medium supplemented with corn steep liquor (SM-BM-CSL) and basal medium supplemented with synthetic nutrients (SM-BM-SN).



**Figure 1** Simulated (line) and experimental (symbols) profile of biomass (■), lipid production (▼) and sugar consumption (●) on *Mucor circinelloides* URM 4182 cultivation in: (A) sugarcane molasses-based media (SM-BM), (B) SM-BM supplemented with synthetic nutrients (SM-BM-SN), and (C) SM-BM supplemented with corn steep liquor (SM-BM-CSL).

Upon analyzing Figure 1, it becomes evident that the model is well-suited for predicting the dynamic behavior of the process over time across the various conditions studied. The supplementation of sugarcane molasses-based media has been empirically

demonstrated to significantly enhance lipid production. Notably, the culture condition incorporating synthetic nutrients (SM-BM-SN) exhibited the highest lipid yield among the tested conditions. In the context of process kinetics, the estimated model parameters for each experimental condition, along with the corresponding sum of squares of the residuals, are detailed in Table 1.

**Table 1** Estimated parameters,  $Y_{X/P}$  values and minimized values (RSS) for the model fit.

Conditions	RSS* ( $\text{g}^2 \text{L}^{-2}$ )	$Y_{X/P}$ ( $\text{g} \times \text{gP}^{-1}$ )	$k_1$ ( $\text{h}^{-1}$ )	$k_2$ ( $\text{L} \text{g}^{-1}$ )
SM-BM	0.046	5.061	0.0814	0.0144
SM-BM-SN	0.039	3.482	0.0849	0.0208
SM-BM-CSL	0.046	5.370	0.0886	0.0234

\*RSS: residual sum of squares.

In addition to the visual criteria, the low residual sum of squares (RSS) values indicate a strong fit of the model to the experimental data. The condition that yielded the highest biomass production was the cultivation with SM-BM-CSL, followed by SM-BM-SN, and lastly SM-BM (Figure 1). Given that the constant  $k_1$ s related to the yield of substrate to cells, a decrease in this value signifies a lower amount of biomass produced, further validating the model's effectiveness. The values obtained for the constant  $k_2$  suggest that a higher magnitude of this constant corresponds to faster substrate consumption, aligning with the kinetic behaviour observed experimentally. Therefore, the estimated parameters of the model were consistent with the dynamic behaviour of the process. A similar behaviour was documented in the studies conducted by Bento et al. (2020)<sup>1</sup> and Bento et al. (2019)<sup>3</sup>.

## 4 CONCLUSION

The substrate, product (lipids) and cell concentration profiles, which characterize the process kinetics, were mathematically modeled, allowing a more complete study of the process. The proposed model showed an excellent fit to the experimental data and the constants estimated for adjusting the model were consistent with the process behavior under different conditions. The cultivation with the addition of synthetic nutrients exhibited the highest lipid production, a fact corroborated by the lower  $Y_{X/P}$  value obtained through modeling.

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