EFFECT OF LIPID SOURCE CONCENTRATION ON WHOLE CELL LIPASE PRODUCTION AND KINETIC EVALUATION

Willian de S. M. Reis[1]*, Jorge H. V. Santos[2], Ana K. F. de Carvalho[3] & Ernandes B. Pereira[4]

[1] *Department of Biotechnology/ Engineering School of Lorena-USP, Lorena (SP), Brazil.*
[2] *Chemical Engineering, Department of Chemical Engineering/ Engineering School of Lorena-USP, Lorena (SP), Brazil.*
[3] *Department of Basic and Environmental Sciences / Engineering School of Lorena-USP, Lorena (SP), Brazil.*
[4] *Faculty of Pharmaceutical Sciences, Federal University of Alfenas, Alfenas (MG), Brazil.*
* *willian.matias@usp.br*

ABSTRACT

The present study aimed to evaluate different concentrations of olive oil in the cultivation of *Rhizopus oryzae* for the production of whole cell lipases and to evaluate the kinetic parameters for cultivation at each oil concentration. The results indicated that the best condition for the production of whole cell lipases was with 3.0% oil in 72 hours of cultivation, achieving activity values of 204 U/g and 28.76 g/L of cellular biomass. The kinetic parameters obtained under this condition were a μmax of 0.018 h^{-1} and a μ_p of 0.65 U.L/g^2.h.

Keywords: mycelium-bound lipase; biotransformation;

1 INTRODUCTION

The production of free fatty acids (FFA) by traditional chemical industries is conducted under harsh process conditions of hydrolysis, such as high temperatures (250ºC) and pressures (50 bar), and is known as the Colgate-Emery Process. Under these operating parameters, separation and purification steps are required because undesirable oxidation, dehydration, and interesterification reactions occur[1]. As an alternative, the use of enzymes in the hydrolysis process of vegetable oils has shown promising results. Among the advantages over the traditional method are moderate conditions of temperature and pressure, easier purification and separation of the final product, and reduced energy costs[2].

Lipases can be produced by animal, microbial and vegetable fonts, although for industrial purposes, the microbial lipases are the most commonly used. The industrial demand searches for the use of immobilized enzymes, since they help to reduce costs on the purification and separation process when in comparison with the enzyme in its free form[1]. The lipases found in the inter-membrane space of cells are naturally immobilized with the active enzymatic mechanism that aids in the processes of extraction and isolation. These whole cells, such as the mycellium-bound lipases associated with fungal biomass, still have catalytic activity. Therefore the major advantages of using whole cells as catalysts include their stability, due to the environment of the enzyme being unaltered during the process, the high yields and enantiomeric excesses in the main reaction[3,4].

The growth and the metabolic activities of organisms are responses to the surrounding physicochemical environmental conditions. Hence, to ensure high lipolytic activity within the cells, it is necessary to optimize the cultivation medium conditions. Variables such as pH, temperature, the concentration of fatty acids of the oil as an inducing agent, and incubation period directly affect cell growth and cellular activity[1,2]. The present study aimed to evaluate different concentrations of olive oil in the cultivation of *Rhizopus oryzae* for the production of whole cell lipases and to evaluate the kinetic parameters for cultivation at each oil concentration.

2 MATERIAL & METHODS

Materials: The strain used was *Rhizopus oryzae* CCT3759 obtained from the André Tosello Tropical Research and Technology Foundation (Campinas, SP, Brazil). To obtain culture spores, fungal cells were previously inoculated on Sabouraud agar medium under aseptic conditions.

Culture Medium and Experimental Conditions and Variation of Olive Oil in the production of lipases: A modified medium described by Reis et al. (2022) was used. The olive oil concentration was analyzed at 1.5%, 3%, and 6% (v/v), all of which were previously autoclaved (121 °C for 15 minutes). Cultivations were made in 250 mL-Erlenmeyer flasks containing 100 mL of autoclaved medium and inoculated with a suspension of 1×10^6 spores at 30 °C and orbital shaking at 180 rpm for 120 hours. The fermented broth and the resulting biomass was quantified for hydrolytic activity. Subsequently, the fungal biomass was stored at 4 °C until use[1,2].

Evaluation Parameters in the Production of Lipase: In each culture cycle, the production of mycelium-bound lipase was evaluated based on dry biomass concentration (g/L) and hydrolytic activity (U/g) (HA) using the olive oil emulsion hydrolysis method described by previous studies5, productivity (U/L.h), and total biomass activity. Enzymatic activity (U/g) is defined as the amount of dry biomass or culture broth required for the release of 1 μmol of FFA per minute under experimental conditions and is calculated using Eq. (1). Productivity was defined as the units of hydrolytic activity produced per liter of cultivation6, calculated using Eq. (2), and the total biomass activity was defined as the total activity of all produced biomass, according to Eq. (3).

$$Enzyme\ activity\ \left(\frac{U}{g}\right) = \frac{(V_a - V_b)\cdot M \cdot 1000}{t \cdot m} \quad (1)$$

Where: V_a = volume of NaOH spent in the sample titration (mL); V_b= volume of NaOH spent in blank titration (mL); M = molarity of the NaOH solution (mol/L); t = reaction time (min); m = mass dry biomass or fermentation broth (g).

$$Productivity\ \left(\frac{U}{L.h}\right) = \frac{HA * X_{DBC}}{t} \quad (2)$$

$$Total\ Biomass\ Activity = HA * m \quad (3)$$

Where: *HA* = hydrolytic activity (U/g); X_{DBC} for dry biomass concentration (g/L); *t* for time; m = mass of dry biomass (g)

Evaluation of Kinetic Parameters: For the kinetic parameters, the experimental data were fitted using Excel (Microsoft, Redmond, USA), employing polynomial functions. The instantaneous rates of cell growth (dX/dt) and lipase production (dP/dt) were calculated using Scilab 5.5.2 (Scilab Enterprises, Versailles, France). Based on these rates and the biomass concentration, the values of the specific growth rates (μ_X) and product formation rates (μ_P) were obtained under each experimental condition according to Eqs. 4 and 5.

$$\text{Specific Cell Growth Rate} = \mu_{max}\ (h^{-1}) = \frac{1}{X} * \frac{dX}{dt} \quad (4)$$

$$\text{Specific Product Formation Rate} = \mu_P\ \left(\frac{U.L}{g^2.h}\right) = \frac{1}{X} * \frac{dP}{dt} \quad (5)$$

3 RESULTS & DISCUSSION

The results in Figure 1 show that there is greater lipase retention in the biomass mycelium (Figure 1a) than in the fermentation broth (Figure 2a), as the hydrolytic activities of the fermentation broth were lower than 30 U/g. The extracellular lipase activity produced by Rhizopus oryzae did not show an increase in average hydrolytic activity values with the increase in olive oil concentration in the culture medium, indicating the characteristic of this fungal strain to produce lipases more efficiently bound to the fungal mycelium. Nevertheless, the highest extracellular lipase production was achieved in the culture with 3.0% oil at 72 hours of cultivation, with an activity of 27.94 ± 1.16 U/g.

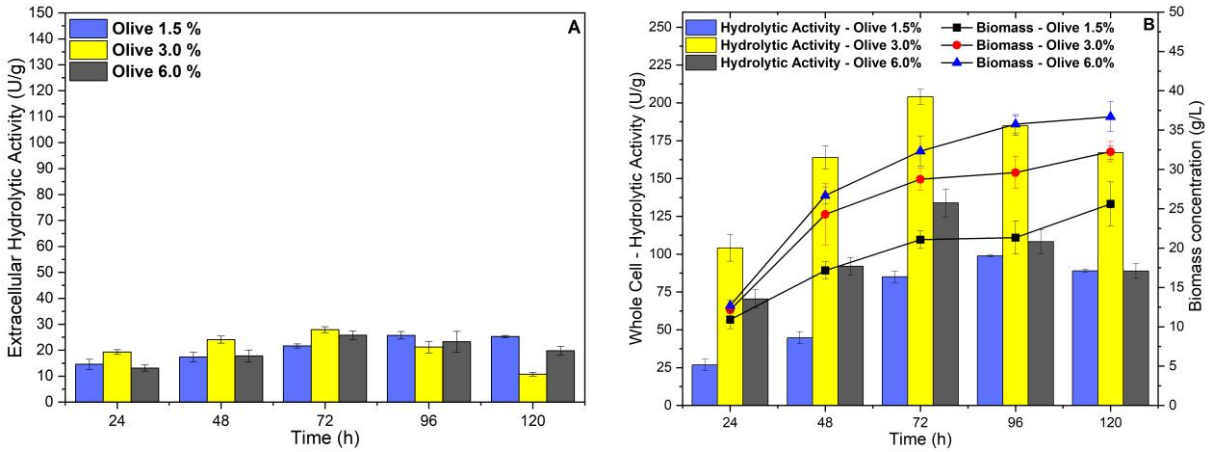

Figure 1 Assessment of olive oil concentrations of 1.5%, 3.0% and 6.0% in: (A) Extracellular Hydrolytic Activity (U/g) and (B) Whole Cells - Hydrolytic Activity (U/g).

The concentration of olive oil in the culture medium influenced the production of lipases from intact cells and biomass production. Regarding cell production, the increase in oil concentration promoted an increase in cell production at all evaluated cultivation times, with the highest biomass production values at the end of 120 hours of cultivation, reaching values of 25.62 ± 2.78, 32.26 ± 1.28, and 36.69 ± 1.91 g/L for olive oil concentrations of 1.5%, 3.0%, and 6.0%, respectively. Among the evaluated oil concentrations, 3.0% promoted the highest production of lipase activity from intact cells at 72 hours of cultivation, with an activity of 204 U/g and a biomass production of 28.76 g/L.

For the concentration of 1.5% olive oil, the highest productivity (Figure 2a) was achieved within the first 24 hours, with a continuous decrease throughout the cultivation period, going from 38.67 to 1.45 U/L.h by the end of the evaluated period. With 6.0% oil, higher enzymatic productivity was achieved, with productivity increasing up to 72 hours, reaching 60.11 U/L.h. However, with a lower concentration of olive oil, higher lipase productivities were obtained, with values ranging from 81.48 to 93.56 U/L.h between 24 and 72 hours. These results suggest that for higher lipase productivity from intact *Rhizopus oryzae* cells, the periods between 48 and 72 hours are the most suitable for cultivation. Furthermore, the use of 3.0% olive oil proved to be the most appropriate concentration to obtain biomass with high catalytic activity. This can also be seen in Figure 2b, where the highest total biomass activity was 586.64 U. Optimal productivity conditions were also observed between 48 and 72 hours of cultivation, as demonstrated by the results of previous studies[1,2].

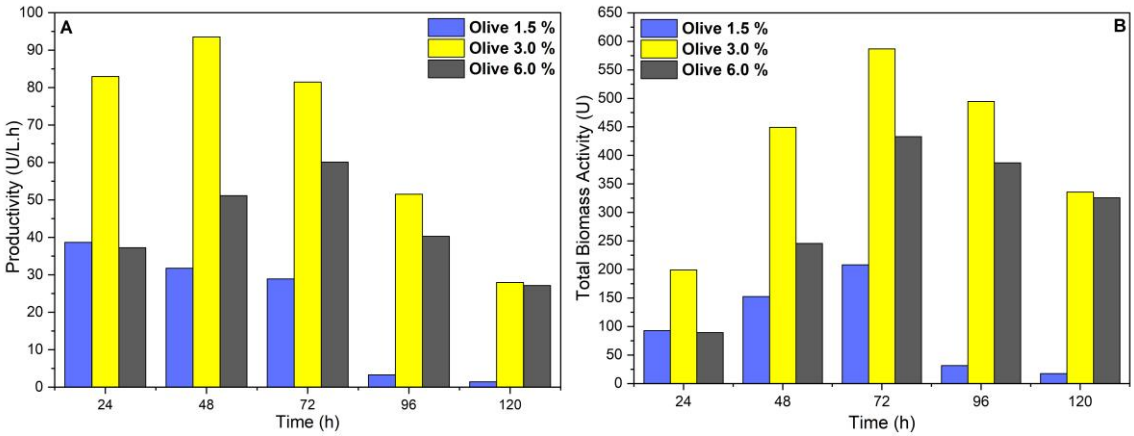

Figure 2 Assessment of olive oil concentrations of 1.5%, 3.0% and 6.0% in: (A) Productivity (U/L.h) and Total Biomass Activity (U).

The kinetic parameters (Table 1) presented show how different concentrations of olive oil affect microbial growth and lipase production. For 1.5% olive oil, the lowest microbial growth rate among the concentrations studied is observed, indicating potential limitation of the microorganism due to low oil content. Lipase production (µp) is also relatively low, suggesting a limited metabolic response. In contrast, the 3.0% concentration of olive oil shows a significant increase in both µmax and µp. This suggests more favorable conditions for microorganism growth, likely due to increased nutrient availability (olive oil). At 6.0% olive oil, there is a slight increase in µmax, indicating growth is still possible, but lipase production (μ_p) decreases compared to the 3.0% concentration, suggesting possible negative regulation or inhibition at higher olive oil concentrations. These results indicate that olive oil concentration directly impacts microbial growth and lipase production. While higher concentrations may promote greater growth, lipase production may not increase proportionally, indicating an optimal point where both growth and lipase production are achieved.

Table 1 Evaluation of the kinetic parameters μ_{max} and μ_p in olive oil concentrations of 1.5%, 3.0% and 6.0%.

Kinetic Parameter	Olive Oil Concentration		
	Olive 1,5%	Olive 3,0%	Olive 6,0%
μ_{max} (h^{-1})	0,014	0,018	0,02
μ_p ($U.L/g^2.h$)	0,15	0,65	0,35

4 CONCLUSION

The results of this study demonstrate the influence of the concentration of the lipolytic source on the production of lipases by intact cells of *Rhizopus oryzae*. An intermediate concentration of olive oil (3.0%) promoted the production of biomass with high lipolytic activity, indicating that at this oil concentration, cellular metabolism is favored for the production of mycelium-bound lipase and satisfactory biomass production.

REFERENCES

1 MATIAS, A. B., REIS, W.S.M. Reis, COSTA-SILVA, T. A., BENTO, H. B.S., CARVALHO, A. K.F. de, PEREIRA, E. B. (2023). Catalysis Communications, Volume 184.
2 REIS W.S.M., MATIAS A.B, MENDES A.A., CASTRO H.F. de, PEREIRA E.B. (2022). Catal Lett. 152:1–11.
3 CORTEZ, D.V, CASTRO, H. F. de, ANDRADE, G.S.S.. Quim Nova 40, 2017 85–96.
4 LIN, B., TAO, Y. 2017. Microb Cell Fact 16, 106.
5 MAROTTI, B.S., CORTEZ, D.V., GONÇALVES,D.B. et al., 2017. Quim Nova, 40:427–435
6 WANG D, ZHU Z, WANG X et al., 2015. Process Biochem, 50:2019–2028.

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