Exploring the potential of novel *Geotrichum candidum* CN-Y7142 for lipase production

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ABSTRACT

This study investigates the lipase production by the newly isolated yeast *Geotrichum candidum* CN-Y7142 under controlled fermentation conditions. The microorganism demonstrated high efficiency in glucose consumption, achieving 96.49% consumption after 96 hours of fermentation. Lipase production peaked at 130 $U.L^{-1}$ at 48 hours and remained high at 72 and 96 hours. Notably, *G. candidum* CN-Y7142 continued to produce lipase even after glucose depletion, suggesting the utilization of soybean oil triglycerides as an alternative carbon source. The pH of the fermentation medium decreased from 5.6 to 4.5 in the first 24 hours and then stabilized. The ability of *G. candidum* CN-Y7142 to sustain lipase production under low glucose conditions underscores its versatility and industrial potential. These findings are promising for optimizing fermentation processes aimed at efficient and economically viable lipase production, contributing to more sustainable bioprocesses in industrial biotechnology.

Keywords: Lipase production. *Geotrichum candidum*. Fermentation kinetics. Enzymatic activity. Industrial biotechnology.

1 INTRODUCTION

Lipases are versatile enzymes present in animals and plants, responsible for catalyzing the hydrolysis of triglycerides into free fatty acids, diacylglycerols, monoacylglycerols, and glycerol. Not only are these reactions crucial for various biological processes, but they are also reversible, allowing the synthesis of acylglycerols from fatty acids and glycerol at the oil-water interface. Lipase is an enzyme that plays a crucial role in several industrial applications, including the production of biodiesel, the manufacture of detergents, and processes in the food industry, due to its ability to catalyze the hydrolysis of triacylglycerols into glycerol and free fatty acids[1].

The study of lipase production by microorganisms, such as *Geotrichum candidum sp*, has gained prominence due to the ability of this fungus to produce high concentrations of lipase under controlled fermentation conditions. The fungus studied is a filamentous species that is being widely recognized for its essential role in cheese production and in various biotechnological applications. *Geotrichum candidum* plays a central role in the food industry, especially in cheese production, and has the potential for significant contributions in several areas of biotechnology[2]. The in-depth understanding of its genetic and metabolic characteristics continues to drive research and practical application of this versatile fungus.

Kinetic analysis of these fermentation processes is essential to understand and optimize cultivation conditions, substrate intake, cell growth, and enzyme production. Through this analysis, it is possible to identify the phases of cell growth, metabolic adaptations, and the relationship between nutrient consumption and enzyme production, allowing precise adjustments in the fermentation process[3]. Understanding kinetic parameters, such as cell growth rates and enzyme production, allows for precise adjustments in the fermentation process, improving productivity and reducing costs.

The kinetic approach of this work involved the detailed analysis of variables such as glucose consumption, cell growth, and enzymatic activity over the fermentation time. The collection and analysis of data at regular intervals allowed the construction of detailed profiles of these variables, enabling the identification of the maximum points of lipase production, glucose consumption and optimal cell growth of lipase production. This study provides a basis for future research and industrial applications, demonstrating the potential of *Geotrichum candidum CN-Y7142* in biotechnology.

2 MATERIALS & METHODS

Microorganism: The new strain of *Geotrichum candidum CN-Y7142* used in this study was provided by the Collection of Microorganisms, DNA, and Cells of the Department of Microbiology at the Federal University of Minas Gerais (UFMG).

Inoculum and fermentation condition: the fermentation medium was composed of 20 $g.L^{-1}$ of pure glucose, 5 $g.L^{-1}$ of yeast extract, 10 $g.L^{-1}$ of malt extract and 2 $g.L^{-1}$ of sodium monobasic phosphate. As an inducer for lipase production, soybean oil was added at a concentration of 2% w/v in the medium. The cultivation media were inoculated using the agar-inoculum cut technique according to the methodology optimized by Maldonato et al., (2014)[4]. Fermentation occurred, in duplicate, at 30°C with stirring at

150 rpm for a total duration of 96 hours, with samples collected at time intervals to determine cell growth profiles, glucose consumption, lipase enzymatic activity, and pH variation.

Consumption of glucose and cell growth: the glucose consumption curve was constructed by High Precision Liquid Chromatography (HPLC) analysis of the samples[5]. The cell growth quantified by gravimetry, a technique that involves the analysis of the dry mass of the cells present in the fermentation medium.

Enzymatic activity: to determine the enzymatic activity of lipase, 1 mL of fermentation medium was added to 5 mL of emulsion prepared with 25% w/w of olive oil, 7% w/w of gum arabic and 2 mL of phosphate buffer solution pH 7.0. After 10 minutes, the reaction was interrupted with the addition of 10 mL of acetone:ethanol solution (1:1). The mixture was then titrated with 0.05 M NaOH to neutralize the released fatty acids[6]. The enzymatic activity of lipase ($U.L^{-1}$) was calculated as follows:

$$A(U/L) = \frac{(Va - Vb) \times N}{t \times Vc}$$

Where: A: is the enzymatic activity in $U.L^{-1}$; Va: is the volume of titrant used in the sample in L; Vb: is the volume of titrant used in the blank in L; Vc: is the volume of sample added in L; N: is the normality of the titrant in eq/L; t: is the reaction time in minutes.

Fermentation parameters: the conversion factors in mass of cells produced per mass of glucose consumed ($Y_{x/s}$), enzymatic activity produced per mass of glucose consumed ($Y_{p/s}$) and lipase expression with respect to time (Q_p)[3] were determinate. From the experimental data, equations describing the behavior of glucose consumption, cell growth and lipase production were adjusted. The kinetics parameters were calculated according to LE DUY and ZAJIC (1973).

3 RESULTS & DISCUSSION

Figure 1 provides valuable information on the growth, glucose consumption, and lipase production by *Geotrichum candidum CN-Y7142.* The fungus exhibited a cell growth of 4.05 $g.L^{-1}$ in 24 hours and 8.08 $g.L^{-1}$ after 96 hours of fermentation. Macedo (2020) conducted a study characterizing two strains of *Geotrichum sp.* and, using glucose as a carbon source, obtained a final cell concentration of 8 $g.L^{-1}$, corroborating the results obtained in this work[7].

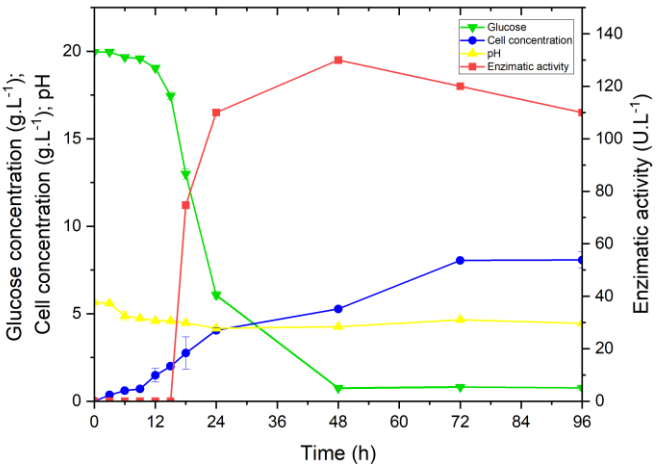

Figure 1 – Glucose consumption, pH variation, cell growth and lipase activity of *Geotrichum candidum* CN-Y7142C cultived in semi-defined medium.

Observing the experimental data presented in Figure 1, it is possible to identify the glucose consumption curve profile over the 96 hours. The fermentation started with 20 $g.L^{-1}$ of glucose and within 24 hours, the concentration reached 6.07 $g.L^{-1}$, resulting in a consumption of 69.6% of the carbon source. After 48 hours, the glucose concentration reached 0.75 $g.L^{-1}$ and remained almost constant until the end of the fermentation. Evaluating the onset of lipolytic activity in the fermentation medium, it is interesting to note that the first samples indicating lipase production coincided with at least 50% glucose consumption in the medium. This indicates that enzyme production is sensitive to the fermentation medium's composition and can be favored or inhibited by the substrate's concentration or source.

In terms of lipase activity obtained, the initial production was observed at 18 hours of fermentation with an activity of 74.7 $U.L^{-1}$, reaching a maximum value of 130 $U.L^{-1}$ at 48 hours. Maldonato (2006) achieved a maximum activity of 18 U/mL in 48 hours using soybean oil as an inducer for lipase production[8]. Additionally, Figure 1 shows that the pH of the fermentation medium decreased from 5.6 to 4.5 in the first 24 hours and remained almost constant for the rest of the fermentation process. Studies indicate that maintaining a pH between 4 and 7 enhances lipase stability, emphasizing the importance of pH control for optimizing enzyme production[4,8].

Table 1 presents the fermentative parameters of *Geotrichum candidum* CN-Y7142, highlighting that the cell yield relative to consumed glucose ($Y_{X/S}$) was 0.42 $g_{cell}/g_{glucose}$ after 96 hours. The production yield of lipase per mass of consumed glucose (Yp/s) was calculated to be 5.51 $U.L^{-1}.g_{glucose}^{-1}$, demonstrating high production of both biomass and lipase.

Table 1 fermentative and kinetics parameters of the *Geotrichum candidum* CN-Y7142 cultived in semi-defined medium.

Parameters		Time (h)
$P_{lipase máx}$ ($U.L^{-1}$)	130	48
Q_p ($U.L^{-1}.h^{-1}$)	1,15	96
$\mu_{x\ máx}$ (h^{-1})	0,21	10
$\mu_{s\ máx}$ ($g_{glucose}.g_{cell}^{-1}.h^{-1}$)	2,10	7
$\mu_{p\ máx}$ ($U.g_{cell}^{-1}.h^{-1}$)	3,86	16
$Y_{p/s}$ ($U.L^{-1}.g_{glucose}^{-1}$)	5,51	96
$Y_{x/s}$ ($g_{cell}/g_{glucose}$)	0,42	96
Glucose consumption (%)	96,49	96

The maximum specific cell growth rate ($\mu_{x\ máx}$) of 0.21 h^{-1} was observed at 10 hours of fermentation (Table 1), a substrate consumption rate ($\mu_{s\ máx}$) of 2.10 $g_{glucose}.g_{cell}^{-1}.h^{-1}$ at 38 hours, and a lipase production rate ($\mu_{p\ máx}$) of 3.86 $U.g_{cell}^{-1}.h^{-1}$ at 16 hours. These parameters provide a comprehensive overview of the fermentative behavior of *Geotrichum candidum* CN-Y7142, highlighting its efficiency in glucose consumption and lipase production. Optimizing these parameters could be essential for enhancing the industrial production of lipase by this microorganism[9].

4 CONCLUSION

This study demonstrates that *Geotrichum candidum* CN-Y7142 is a highly efficient microorganism for industrial lipase production due to its effective glucose utilization and high lipase activity. The kinetic parameters obtained provide valuable insights for optimizing fermentation processes and enhancing enzymatic yield, showcasing its potential in various biotechnological applications. The yeast's ability to produce lipase even after glucose depletion, likely by utilizing triglycerides from soybean oil, underscores its versatility and industrial viability. Furthermore, maintaining an acidic pH during fermentation significantly favored enzyme production, emphasizing the importance of environmental control. These findings highlight *G. candidum* CN-Y7142 as a promising candidate for industrial applications.

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