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Production and characterization of a β-glucosidase by *Aspergillus* sp. in solid-state cultivation of agro-industrial residues

Produção e caracterização de uma β-glicosidase por *Aspergillus* sp. em cultivo em estado sólido de resíduos agroindustriais

Producción y caracterización de una β-glucosidasa mediante *Aspergillus* sp. en cultivo en estado sólido de residuos agroindustriales

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RESUMO

Introdução: As β-glicosidases são enzimas celulolíticas que apresentam diversas aplicações industriais, como na produção de biocombustíveis e na indústria de sucos e vinhos.

Objetivo: Avaliar o potencial de aproveitamento de resíduos agroindustriais para a produção de β-glicosidase pelo fungo *Aspergillus* sp. e determinar parâmetros de cultivo visando aumentar a atividade enzimática.

Métodos: Para a produção da enzima, foram avaliados os seguintes parâmetros: tipo de substrato, tempo de cultivo, solução nutriente suplementar, pH da solução nutriente, umidade inicial do substrato e temperatura de incubação do fungo. Na melhor condição encontrada, a enzima foi caracterizada em relação ao pH e temperatura ótimos, bem como à estabilidade a estes fatores.

Resultados: Os valores de atividade da β-glicosidase apresentaram diferença significativa quando o fungo foi cultivado nos substratos compostos por farelo de trigo e bagaço de cana (1:1 p/p), farelo de trigo e bagaço de malte (1:1 p/p) e na mistura dos três substratos (1:1:1 p/p), em relação ao cultivo em farelo de trigo e na mistura de bagaço de cana e bagaço de malte (1:1 p/p). A atividade enzimática foi mais elevada nas seguintes condições de cultivo: solução nutriente composta por NH_4NO_3, $MgSO_4.7H_2O$ e $(NH_4)_2SO_4$ (0,1%) com pH 4,5 e 5,5, temperatura de incubação do fungo a 35 °C, com umidade inicial do substrato em 65%. A enzima apresentou maior atividade na faixa de pH entre 4,5 e 5,5, e estabilidade em uma ampla faixa de pH (3,0 a 8,0). A temperatura ótima foi de 65 °C e a enzima apresentou de estabilidade superior a 70% por 1h, até 55 °C.

Conclusão: A utilização de resíduos agroindustriais proporcionou elevada produção de β-glicosidase pelo fungo, com a enzima apresentando características com potencial de aplicação industrial.

Palavras-chave: Celulase; Fungo; Biodegradação.

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ABSTRACT

Introduction: β-glucosidases are cellulolytic enzymes with various industrial applications, such as in the production of biofuels and in the juice and wine industry.

Objective: To evaluate the potential use of agro-industrial residues to produce β-glucosidase by the fungus *Aspergillus* sp. and to determine cultivation parameters aimed at increasing enzymatic activity.

Methods: The following parameters were evaluated for enzyme production: type of substrate, cultivation time, supplementary nutrient solution, pH of the nutrient solution, initial substrate moisture, and incubation temperature of the fungus. Under the best condition found, the enzyme was characterized in relation to optimal pH and temperature, as well as stability to these factors.

Results: The β-glucosidase activity values showed significant differences when the fungus was cultivated on substrates composed of wheat bran and sugarcane bagasse (1:1 w/w), wheat bran and malt bagasse

(1:1 w/w), and the mixture of the three substrates (1:1:1 w/w), compared to cultivation on wheat bran and the mixture of sugarcane bagasse and malt bagasse (1:1 w/w). The highest enzymatic activity was observed under the following cultivation conditions: nutrient solution composed of NH_4NO_3, $MgSO_4{\cdot}7H_2O$, and $(NH_4)_2SO_4$ (0.1%) with pH 4.5 and 5.5, fungus incubation temperature at 35°C, with initial substrate moisture at 65%. The enzyme showed highest activity in the pH range between 4.5 and 5.5, and stability over a wide pH range (3.0 to 8.0). The optimal temperature was 65°C, and the enzyme exhibited stability above 70% for 1 hour up to 55°C.
Conclusion: The use of agro-industrial residues resulted in high production of β-glucosidase by the fungus, with the enzyme exhibiting characteristics with potential industrial application.
Keywords: Cellulase; Fungus; Biodegradation.

RESUMEN

Introducción: Las β-glucosidasas son enzimas celulolíticas que tienen diversas aplicaciones industriales, como en la producción de biocombustibles y en la industria de zumos y vinos.
Objetivo: Evaluar el potencial de aprovechamiento de residuos agroindustriales para la producción de β-glucosidasa por el hongo *Aspergillus* sp. y determinar parámetros de cultivo orientados a incrementar la actividad enzimática.
Métodos: Para la producción de la enzima se evaluaron los siguientes parámetros: tipo de sustrato, tiempo de cultivo, solución nutritiva suplementaria, pH de la solución nutritiva, humedad inicial del sustrato y temperatura de incubación del hongo. En las mejores condiciones encontradas, la enzima se caracterizó en relación con el pH y la temperatura óptimos, así como con la estabilidad a estos factores.
Resultados: Los valores de actividad de la β-glucosidasa mostraron una diferencia significativa cuando el hongo se cultivó sobre sustratos compuestos por salvado de trigo y bagazo de caña (1:1 p/p), salvado de trigo y bagazo de malta (1:1 p/p) y en la mezcla de los tres sustratos (1:1:1 p/p), en relación al cultivo en salvado de trigo y en la mezcla de bagazo de caña y bagazo de malta (1:1 p/p). La actividad enzimática fue mayor en las siguientes condiciones de cultivo: solución nutritiva compuesta por NH_4NO_3, $MgSO_4.7H_2O$ y $(NH_4)_2SO_4$ (0.1%) con pH 4.5 y 5.5, temperatura de incubación del hongo a 35 °C, con humedad inicial del sustrato a 65%. La enzima mostró mayor actividad en el rango de pH entre 4,5 y 5,5, y estabilidad en un amplio rango de pH (3,0 a 8,0). La temperatura óptima fue 65 °C y la enzima mostró una estabilidad superior al 70% durante 1 hora, hasta 55 °C.
Conclusión: El uso de residuos agroindustriales proporcionó una alta producción de β-glucosidasa por parte del hongo, presentando la enzima característica con potencial para aplicación industrial.
Palabras-clave: Celulasa; Hongo; Biodegradación.

INTRODUCTION

Cellulose is the main constituent of plant cell walls and the most abundant source of renewable carbon in nature, making it an important component of urban solid waste, crop residues, animal manure, wood, forest residues, or energy crop residues. Consequently, there is considerable interest in converting biomass formed from industrial agriculture waste into fermentable sugars, which can be utilized in various industrial applications (Taherzadeh; Karimi, 2008; Singhania *et al*., 2010).

The enzymatic hydrolysis of cellulose into glucose requires the synergistic action of at least three different enzymes, including endoglucanases (EC 3.2.1.4), which internally hydrolyze cellulose chains; exoglucanases (EC 3.2.1.91) that attack the non-reducing and reducing ends of cellulose, releasing cellobiose; and β-glucosidases (EC 3.2.1.21) which hydrolyze cellobiose and oligosaccharides, thereby releasing glucose (Yu *et al*., 2007; Garcia *et al*., 2015).

In nature, β-glucosidases play various biochemical, physiological, and nutritional roles in different organisms. From the knowledge of their mechanisms of action, various industrial applications of these enzymes have been developed, such as the hydrolysis of lignocellulose for biofuel production; hydrolysis of glycosides in fruit juices and wines to enhance aroma; synthesis of bioactive aglycones from glycoside conjugates; and production of alkyl glycosides, which are useful ingredients in cosmetics and detergents (Godse *et al*., 2021).

To produce cellulolytic enzymes, solid-state fermentation (SSF) is an efficient process. This process involves minimal free water activity but sufficient moisture in the substrate to support microbial growth (Hansen *et al*., 2015).

Regarding the enzyme β-glucosidase, many studies on enzyme production in SSF have been conducted with fungi such as *Aspergillus*, *Penicillium*, and *Trichoderma*, utilizing various agro-industrial residues such as wood, corn cobs, rice straw, sorghum, sugarcane bagasse, and wheat bran, thereby adding value to these materials (Gao *et al*., 2008; Asgher *et al*., 2016; Magwaza; Amobonye; Pillai, 2024).

The use of agricultural residues for enzyme production allows for reducing overall production costs. Moreover, the application of these residues in bioprocesses has become important from an environmental perspective, reducing problems related to improper handling and consequent environmental damage (Santos *et al*., 2016; Devi *et al*., 2022; Shanmugam *et al*., 2022). Thus, agro-industrial residues represent an alternative that has been gaining ground in the world market because it contains in its composition cellulosic raw materials with good availability, low lignin content, shorter growth cycles, and its use indicates economic and environmental responsibility (De Melo *et al*., 2023).

A successful strategy for producing cellulolytic enzymes includes the selection of microorganisms, understanding the basic physiology of cellulolytic microorganisms, and improving cultivation parameters (Behera; Ray, 2016). Besides the substrate, β-glucosidase production is influenced by cultivation conditions such as incubation time, pH, incubation temperature, and supplementary nutrient sources. Therefore, optimizing cultivation conditions is crucial to ensure maximum enzyme production (Kao *et al*., 2019; Singh *et al*., 2023).

In this context, the study aimed to evaluate the potential production of β-glucosidase by the fungus *Aspergillus* sp. in solid-state fermentation using agro-industrial residues under different cultivation conditions, as well as to characterize the enzyme.

METHODS

Microorganism

The fungus *Aspergillus sp*. used in this study was isolated from soil collected in an agroecological garden at Fazenda Horta Rio Grande, located in the municipality of Fronteira, Minas Gerais, Brazil. The sampling site is situated in the Triângulo Mineiro region at coordinates 20°16′04″ S and 49°11′58″ W.

Solid Nutrient Medium for Subculturing and Maintenance of Colonies

For periodic subculturing and preservation of pure cultures, a culture medium composed of 3% oatmeal (Quaker®) and 1% bacteriological agar was used. The pure cultures were maintained in

cryotubes, under a 20% glycerol solution, in a freezer at -80°C.

Solid-State Cultivation Medium (SSC)

The fungus was cultivated in 250 mL Erlenmeyer flasks containing 5.0g of the following substrates: wheat bran (WB); wheat bran + sugarcane bagasse (WB SB) (1:1 w/w); wheat bran + malt bagasse (WB MB) (1:1 w/w); sugarcane bagasse + malt bagasse (SB MB) (1:1 w/w); and a mixture of these three materials (WB SB MB) (1:1:1 w/w).

The sugarcane bagasse and malt bagasse were obtained from a sugar-energy plant and a brewery, respectively, in the municipality of Frutal/MG. The wheat bran was acquired from local commerce in the same municipality. The substrates were washed, dried at 60°C in a drying oven, and sieved to 10 mesh. Initially, sterilized distilled water with a pH adjusted to 5.0 was added to each substrate in an amount to ensure the initial moisture content of each was 70%.

Pre-inoculum for Solid-State Cultivation

For each SSC cultivation, a pre-inoculum was prepared in a 250 mL Erlenmeyer flask containing 100 mL of medium composed of 3% oatmeal (Quaker®) and 1% agar, with the pH adjusted to 5.5 using HCl. The fungus was inoculated on the surface of this medium by streaking and incubated at 30°C until full growth.

After this period, the microorganism was suspended, with the aid of an inoculation loop, in 150 mL of distilled water and used as the inoculum for each Erlenmeyer flask containing the substrates.

Production of the Enzyme by Solid-State Cultivation

The SSCs were conducted in 250 mL Erlenmeyer flasks, using 5g of each substrate and 2.0 mL of fungus suspension. In the cultivations to evaluate the influence of time on enzyme production, 9.0 mL of sterilized distilled water was initially used to hydrate the medium, at pH 5.0, and the fungus was cultivated at 30°C. Every 24 hours, samples (in triplicate) were taken up to 120 hours.

For each sample, 50 mL of distilled water was added, and the mixture was manually homogenized and then kept under agitation in a shaker (140 rpm) for 20 minutes. After this period, the material was filtered through a nylon fabric disc, centrifuged at 10,000 g for 15 minutes at 5°C, and the supernatant was used to determine enzymatic activities.

Cultivation Parameters

To evaluate the effect of substrate supplementation on enzyme production, the following nutrient solutions were used with the substrate: 1- distilled water; 2- NH_4NO_3, $MgSO_4{\cdot}7H_2O$, and $(NH_4)_2SO_4$ (all at 0.1%); 3- yeast extract at 0.1%, added initially so that the initial moisture content was 70%, with the fungus incubated at 30 ºC. Each solution had its pH adjusted from 4.0 to 6.0 (with 0.5 increments), in a 3x5 factorial design, with the experiment conducted in triplicate.

In evaluating the effect of the initial substrate moisture content, volumes of the nutrient solution (chosen in the previous step) were added along with the inoculum so that the initial moisture content was 60%, 65%, 70%, 75%, and 80%. The cultivation temperatures evaluated were 30°C, 35°C, 40°C and 45°C, in a 5x4 factorial design, also in triplicate.

Characterization of β-Glucosidase

The effect of pH on β-glucosidase activity was determined by incubating 50 µL of enzyme solution in 250 µL of the substrate p-nitrophenyl β-D-glucopyranoside (Sigma®) (4mM), in the following buffers (0.2 M): sodium citrate (pH 3.0), sodium acetate (pH 3.5-5.5), MES (pH 6.0-6.5), HEPES (pH 7.0-7.5), glycine-NaOH (pH 8.0-10.0). To determine the enzyme's stability against pH variations, a final volume of 1.0 mL (crude extract properly diluted in acetate buffer pH 5.0) was incubated at 8°C for 24 hours. After the incubation period, enzymatic activity was measured at the enzyme's optimal temperature and pH.

The effect of temperature on enzymatic activity was determined by varying the temperature between 40 and 80°C (in 5°C increments). The activity assays were performed at the enzyme's optimal pH. Thermal stability was assessed by incubating the enzyme extract for one hour at temperatures ranging from 10 to 80°C, followed by the determination of residual activity under optimal pH and temperature conditions.

Determination of β-Glucosidase Activity

For the determination of β-glucosidase activity, 50 µL of the enzyme extract was added to a mixture of 250 µL of acetate buffer solution (0.1 M, pH 5.0) and 250 µL of 4-nitrophenyl-β-D-glucopyranoside (4 mM) – PNPG, Sigma. The reaction was initially maintained at 60°C for 10 minutes and then stopped by adding 2.0 mL of Na_2CO_3 solution (2M). The released nitrophenol was quantified by spectrophotometry at 410 nm. One unit of enzymatic activity was defined as the amount of enzyme required to release 1.0 µmol of nitrophenol per minute of reaction, using a standard curve obtained with nitrophenol solution at various concentrations.

Statistical Analysis of Data

With the general data obtained from the different treatments applied, an analysis of variance (ANOVA) of the experiments was performed, followed by the application of the Scott-Knott test at a 5% probability level for the means obtained.

RESULTS AND DISCUSSION

Production of β-Glucosidase by *Aspergillus* sp.

It was observed that the substrates which provided significantly higher production of β-glucosidase by the fungus were wheat bran + sugarcane bagasse (48h to 120h of cultivation), wheat bran and malt bagasse (48h and 72h of cultivation), and the mixture of wheat bran, sugarcane bagasse, and sugarcane straw (at 48h, 72h, and 120h of cultivation) (Table 01).

Table 01: Production of β-glucosidase by *Aspergillus* sp. in different substrates and cultivation times.

Substrate	Enzymatic activity ($U.g^{-1}$)				
	Cultivation time (h)				
	24	48	72	96	120
WB	0 Ac	9.3 Ba	9.9 Ba	7.6 Bb	6.5 Bb
WB SB	0 Ab	12.7 Aa	12.5 Aa	12.5 Aa	12.1 Aa
WB MB	0 Ac	13.2 Aa	11.2 Ba	8.9 Bb	8.9 Bb
SB MB	0 Ab	4.1 Ca	4.5 Ca	6.5 Ba	6.3 Ba
WB SB MB	0 Ad	12.5 Aa	14.1 Aa	11.0 Ab	13.4 Aa

Note: WB: wheat bran; WB SB: wheat bran + sugarcane bagasse (1:1 w/w); WB MB: wheat bran + malt bagasse (1:1 w/w); SB MB: sugarcane bagasse + malt bagasse (1:1 w/w); WB SB MB: wheat bran + sugarcane bagasse + malt bagasse (1:1:1 w/w).

Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other according to the Scott-Knott test (5%).

Given these results, the WB SB MB substrate, at a cultivation time of 48 hours, was selected for subsequent experiments to determine different cultivation conditions, as it allowed for the utilization of all three evaluated residues and enzyme production occurred in a short period, resulting in efficiency and lower costs for its production.

Several studies report wheat bran as an efficient substrate to produce fungal cellulases both in

isolation and as part of mixtures with other substrates (Leite *et al.*, 2008; Garcia *et al.* 2015; Brijwani; Oberoi; Vadlani, 2010; Martins; Martins; Martins, 2020). In the present work, it was observed that wheat bran was more efficient when mixed with the other evaluated substrates. When the fungus was cultivated only on wheat bran or in mixtures of substrates without wheat bran, enzymatic activity was significantly lower. According to Magwaza, Amobonye, and Pillai (2024), the mixture of wheat bran with other substrates can significantly increase BGL production. Supporting results were reported by Tiwari *et al.* (2015) in the production of this enzyme by *A. niger* SH3 cultivated in a combination of wheat bran and wheat straw, and by Nishida *et al.* (2018), in the production of BGL by *A. awamori* when cultivated in a mixture of wheat bran and pineapple leaves.

Rajoka *et al.* (2006) report that wheat bran has suitable nutritional properties as a substrate for microbial growth, as it contains balanced amounts of carbohydrates, proteins, fats, fibers, and ash (Ca, Mg, P, K, S), favoring enzyme production.

Effect of Fermentation Parameters on the Production of β-Glucosidase by *Aspergillus* sp.

In the evaluation of the effect of different nutrient solutions supplementing the WB SB MB substrate, after 48 hours of cultivation, on the production of β-glucosidase, it was observed that supplementation with salt solution at pH 4.5 or 5.5, or with yeast extract at pH 5.0, significantly increased enzyme production compared to when only water was used (Table 02). Therefore, for subsequent experiments, cultivation with salt solution supplementation at pH 4.5 was chosen, as at this pH, the risk of fungal cultivation contamination by bacteria is lower.

At pH 4.5, compared to the values found using only water, supplementation with the salt solution increased the β-glucosidase activity value obtained by the fungus by 55.9% (Figure 3), demonstrating the importance of evaluating supplemental nutrient sources for the substrate in enzyme production. This result is similar to that found by Martins, Martins e Martins (2020), who reported that β-glucosidase production by *Myceliophthora heterothallica* was significantly higher when the substrate was supplemented with the same salt solution at pH 4.5.

Table 02: Production of β-Glucosidase by *Aspergillus* sp. on a substrate composed of wheat bran, sugarcane bagasse, and sugarcane straw (1:1:1), supplemented with different nutrient solutions, at different pH values, over 4 days of cultivation. 1- water; 2- NH_4NO_3 at 0.1%; 3- $(NH_4)_2SO_4$ at 0.1%; 4- NH_4NO_3, $MgSO_4{\cdot}7H_2O$, and $(NH_4)_2SO_4$ (all at 0.1%); 5- yeast extract at 0.1%; 6- water (control).

Nutrient solution	Enzymatic activity ($U.g^{-1}$)			
	pH			
	4,5	5,0	5,5	6,0
Water (control sample)	18.4 Ba	16.0 Ba	16.7 Ca	16.5 Aa
Salt solution	28.7 Aa	23.0 Ab	27.3 Aa	16.2 Ac
Yeast extract	18.8 Bb	22.7 Aa	20.1 Bb	15.8 Ac

Note: Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other according to the Scott-Knott test (5%).

The use of supplemental nutrient sources to the substrate is important for fungal growth and enzyme production. Gottschalk *et al.* (2013) report the importance of nitrogen supplementation to the substrate, indicating that inorganic nitrogen sources are generally more easily assimilated by fungi than organic sources, emphasizing that this depends on the element concentration and fungal strain. Martins *et al.* (2019) reported that β-glucosidase activity was significantly higher when the substrate was supplemented with nutrient sources containing Sulfur. Therefore, studying supplemental sources is essential to increasing microbial enzyme production.

The initial pH of the medium has a significant effect on the growth and production of β-glucosidase

by many microorganisms, as it can affect cell permeability and other physiological activities. In solid-state cultivation, most filamentous fungi are known for their ability to grow over a wide pH range due to the buffering capacity of these solid substrates (Pandey, Soccol; Mitchell, 2000; EL-Ghonemy, 2021).

Under the best conditions established in the previous experiments, the effect of the initial moisture content of the substrate (WB SB MB) and the incubation temperature of the fungus on β-glucosidase activity was determined.

The condition that provided the highest enzyme activity, with a statistically significant difference, was incubating the fungus at 35°C with a moisture content of 65%. Under this condition, β-glucosidase activity increased by 71.7% compared to the value before this evaluation (Table 03).

Table 03: Production of β-Glucosidase by *Aspergillus* sp. on a substrate composed of wheat bran, sugarcane bagasse, and sugarcane straw (1:1:1), supplemented with NH_4NO_3, $MgSO_4 \cdot 7H_2O$, and $(NH4)_2SO_4$ (0.1%), at pH 4.5, after 48 hours of cultivation, under different moisture and incubation temperature conditions for the fungus.

Incubation temperature (°C)	Enzymatic activity ($U.g^{-1}$)				
	Initial substrate moisture (%)				
	60	65	70	75	80
30	4.8 Bb	7.2 Cb	8.5 Cb	13.1 Ba	14.9 Ba
35	21.9 Ac	31.6 Aa	25.8 Ab	17.4 Ac	19.8 Ac
40	24.5 Aa	20.3 Ba	11.9 Cb	10.2 Bb	12.1 Bb
45	19.5 Aa	18.5 Ba	17.4 Ba	11.6 Bb	12.1 Bb

Note: Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other according to the Scott-Knott test (5%).

The cultivation temperature of microorganisms in solid-state cultivation is a parameter that affects microbial growth, enzyme production, and the time interval in which these enzymes are obtained. The most appropriate cultivation temperature for obtaining β-glucosidase in the present work (35°C) is similar to the values found by Pirota *et al.* (2016), who reported that the highest β-glucosidase activity produced by *Aspergillus oryzae* was obtained between 35°C and 37°C, and by Elyas *et al.* (2010), who reported that the highest β-glucosidase activity of *Aspergillus* AS58 also occurred with the fungus cultivated at 35°C.

In solid-state cultivation, determining the initial moisture content of the substrate is also an essential factor for fungal growth and enzyme production. When the moisture content is below the required level, nutrient solubility is limited, hindering the effective absorption of nutrients. Conversely, with excess moisture, the substrate particles become more compacted, limiting air diffusion between and around the particles (Hamidi-Esfahani; Shojaosadati; Rinzema, 2004; Rodríguez-Zúñiga *et al.*, 2011). As reported by Yoon *et al.* (2014), moisture values below 60% and above 80% are generally unfavorable for both fungal growth and cellulase production.

Characterization of β-Glucosidase Produced by *Aspergillus* sp.

The optimal pH of β-glucosidase was between 4.5 and 5.5, with activities very close to each other within this pH range, when incubated at 60°C (Figure 5). Thus, the data indicate that the fungus produces a β-glucosidase that can be applied in processes requiring more acidic pH values.

Regarding stability when exposed for 24 hours in buffers without substrate at different pH values, it was observed that the enzyme retains more than 85% of its activity over a wide pH range (3.0 to 8.0). In the optimal pH range (4.5 to 5.5), it retained more than 97.0% of its activity after 24 hours (Figure 01), showing high stability to pH variations.

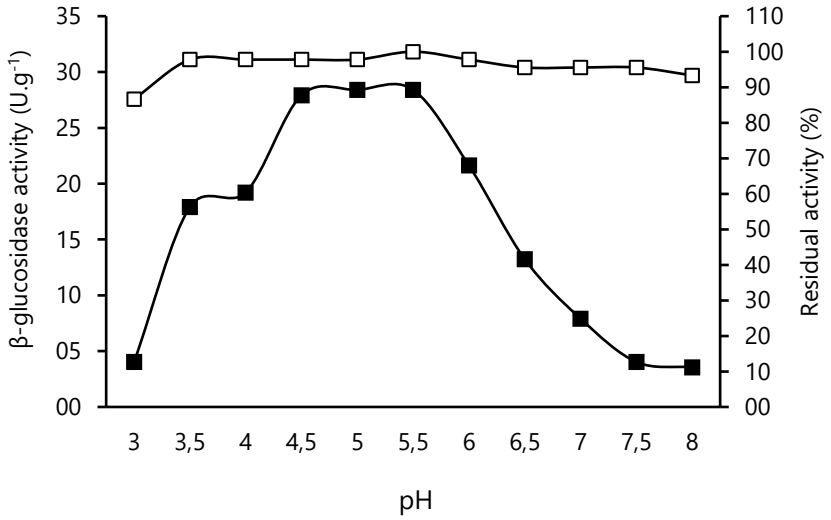

Figure 01: Effect of pH on the activity of β-glucosidase from the fungus *Aspergillus* sp.
–■– optimal pH; –□– pH stability, during 24h, at 8°C.

The optimal pH value of the enzyme from the fungus *Aspergillus* sp. is similar to those described in the literature for fungal β-glucosidases. Baffi *et al.* (2011) report that most β-glucosidases have an optimal pH between 4.0 and 6.0. Garcia *et al.* (2015) reported that the optimal pH of β-glucosidase produced by *Lichtheimia ramosa* was in the range of 5.0 to 6.0, with a peak at pH 5.5. Santos *et al.* (2016) described that the β-glucosidase from the fungus *Gongronella butleri* had an optimal pH of 4.5.

Regarding stability against pH variations, the enzyme from the fungus *Aspergillus* sp. showed high stability (over 90%) over a wide pH range (3.5 to 8.0), maintaining more than 97% of its activity in the optimal pH range.

The activity of β-glucosidase was higher at temperatures of 60°C and 65°C (the temperature at which the highest activity was detected). Beyond this temperature, the activity decreased, especially at 75°C (Figure 02). Regarding thermal stability, the results showed that β-glucosidase maintains stability above 90%, 80%, and 70% when exposed for 1 hour at temperatures of 40°C, 50°C, and 55°C, respectively. On the other hand, at temperatures above 60°C, the activity declined (Figure 02).

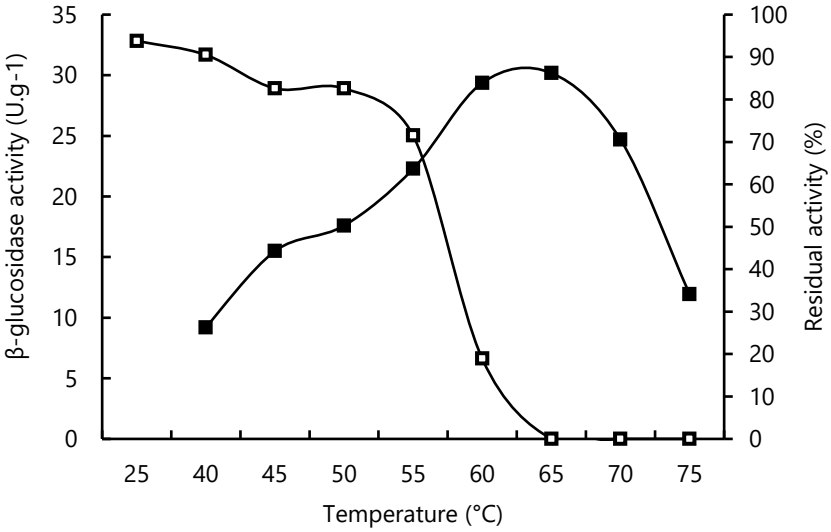

Figure 02: Effect of Temperature on the Activity of β-Glucosidase from the Fungus *Aspergillus* sp.
–■– optimal temperature; –□– stability at different temperatures for 1 hour.

The optimal temperature of β-glucosidase from the fungus *Aspergillus* sp. is above the optimal temperature range for these enzymes produced by different mesophilic fungi. Regarding thermal stability, the presented results are similar to those found for other mesophilic fungi.

Baffi *et al.* (2011) reported that most fungal β-glucosidases have optimal activity at temperatures ranging from 40 to 50°C. El-Ghonemy (2021) emphasizes that high activities of enzymes produced by mesophilic microorganisms are not routinely detected at temperatures above 50°C. On the other hand, Garcia *et al.* (2015) also reported that a β-glucosidase from the mesophilic fungus *Lichtheimia ramosa* showed an optimal temperature of 65°C with thermal stability similar to that presented by the fungus *Aspergillus* sp. in the present study.

At optimal temperature, the fungus *Aspergillus* sp. presented an activity of 30.2 $U.g^{-1}$. When compared with other fungi that produce thermophilic β-glucosidases, it was observed that *Aspergillus* sp. presented a higher activity than that reported by Moretti *et al*. (2012) with the fungus *Myceliophthora thermophila* (22.0 $U.g^{-1}$) in wheat bran. On the other hand, Leite *et al*. (2008) found a higher activity (58.0 $U.g^{-1}$) with *Thermoascus aurantiacus* in this same substrate. According to Acharya and Chaudary (2012), thermophilic cellulases have a wide range of applications. In the food industry, they can be used in the extraction and clarification of fruit juices and olive oil. In the textile industry, they are used to give a faded appearance to jeans. In the production of fuels from lignocellulosic material, they are used in ethanol production. Thus, the high-temperature activity of β-glucosidase from *Aspergillus* sp. is desirable in industrial processes that require cellulolytic enzymes to maintain activity under high temperatures.

CONCLUSION

The use of agro-industrial residues as a substrate provided good production of β-glucosidase by the fungus, with the enzyme exhibiting desirable characteristics from an industrial application perspective, such as good thermal stability and stability over a wide pH range. The optimization of fermentation parameters proved to be fundamental for the increased production of the enzyme.

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