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An Eco-Friendly Production of a Novel and Highly Active Endo-1,4-beta-xylanase from *Aspergillus clavatus*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TMP, RCL and MLTMP conceived and designed the experiments. Authors TMP, ASAS and RCL performed the experiments. Authors TMP, ASAS, TBO, MC and MLTMP analyzed the data. Authors MLTMP, ASAS, RCL, TBO, MC and MLTMP wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Here, we describe a novel way to produce an endo-1,4-beta-xylanase from *Aspergillus clavatus* using paper and pulp industry waste.
Methodology: Optimal *Aspergillus clavatus* NRRL1 cultivation conditions were evaluated using minimal medium with different concentrations (1 to 10%) of paper sludge pretreated with HCl, during different periods (1 to 14 days), with different pH values (3.0; 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0), different temperatures (25, 30, 35, and 40 ºC) and different mixing conditions

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(static and stirring). After that, the enzyme activity was determined by DNS (3,5-dinitrosalicylic acid), protein concentration was quantified by Bradford, SDS-PAGE was performed to evaluate the molecular mass, and TLC observed hydrolysis products.
Results: The enzyme showed a molecular mass of 25 kDa, and its production has been highly improved by optimizing culture conditions. The best activity of this enzyme was obtained when *A. clavatus* was cultivated for 5 days, at 120 rpm, 5% paper sludge, pH 6.0, and 35 ºC. The degradation profile of the beechwood xylan by the crude extract containing the GH11 xylanase showed xylotriose as the main product, but xylotetraose and xylobiose were also produced in significant amounts.
Conclusion: In addition to the fact that this xylanase has the property of producing large quantities of XOS (mainly xylotriose), it has the advantage of being obtained from recyclable waste of the pulp and paper industry. These facts confer great potential for future biotechnological and industrial applications.

Keywords: Aspergillus clavatus; biotechnological and industrial applications; endo-1,4-beta-xylanase; paper sludge; glycosyl hydrolase family.

1. INTRODUCTION

The use of fungal and bacterial enzymes has dominated the industrial sectors due to their advantages, such as cost-effectiveness, shorter production time, and ease of process optimization [1]. In addition, there is appreciation in the utilization of fungi in bioprocesses due to its ability to produce enzymes with different physicochemical characteristics and excellent potential for industrial applications. The production capacity of large-scale enzymes and the facility with which these enzymes are secreted to the external environment are some of the many exciting features found in fungi. Furthermore, fungal enzymes can have several industrial advantages, such as resistance to organic solvents [2] and thermo/pH-stabilities [3,4].

A total of five million tons of biomass are produced per year from different industries as the industry of paper production, which is one of the largest waste producers in the world. Various waste produced from the paper industry could be more beneficial for generating highly added-value products, such as eucalyptus chips, low-quality Kraft pulp, spent sulfite liquors, and pulp and paper sludge [5]. All the by-products obtained by industries worldwide can be used in several processes for high biotechnological and valuable compounds. Some compounds that could be obtained from these wastes are xylitol, prebiotics, biofuels, organic acids, etc. Also, these kinds of residues can be used for microorganisms' cultivation to generate enzymes with great industrial interest [6]. The paper sludge composition consists mainly of 75% cellulose, 15% lignin, 8% hemicellulose, 2% ash, and extractives [7]. The xylan is a xylopyranose with β-1,4 linkages that constitute the biosphere's hemicellulosic polysaccharide [7].

Many hydrolytic enzymes can carry out xylan biodegradation. Therefore, using such enzymes as catalysts in industrial processes has become a crucial way to obtain high-quality products with clean technologies in harmony with the market's technological needs and environmental preservation [8]. Among the most widely used are endo-β-1,4-xylanases, commonly referred to as xylanases, enzymes that randomly break the glycosidic bonds in the main linear chain of xylan. For this, products are released with retention of the anomeric configuration of the substrate and the formation of a covalent intermediary xylosyl-enzyme. Then comes subsequent hydrolysis via oxocarbenium ion-like transition states, at the active site, from a pair of carboxylic acids [9,10].

These types of enzymes are known to have potential in different applications, such as in the hydrolysis of lignocellulose from raw materials [11], in preparation for animal feed [12], bleaching of wood pulp without chlorine [13], and hydrolysis of brewer's spent grain [14]. Also, using xylanases for xylooligosaccharides production as prebiotics has gained so much interest recently [15].

XOS oligomers are made of xylose sugar units, naturally present in fruits, vegetables, bamboo, honey, milk, and can be obtained on an industrial scale from materials rich in xylan [10,16]. In addition, some XOSs, with 2-10 units of polymerization degree, have shown significant

effects as prebiotic. Therefore, these XOS have great potential to be applied as ingredients in food and pharmaceutical [17].

Therefore, this work aimed to standardize the *Aspergillus clavatus* cultivation on paper sludge as a carbon source to obtain high levels of the endo-1,4-β-xylanase at the crude extracellular extract containing a low concentration of other enzymes (ß-glucosidase and ß-xylosidase), as previously reported by screening [18]. Moreover, this work's objective was to evaluate the hydrolysis profile of *A. clavatus* xylanase with xylan as substrate, looking for xylooligosaccharides production.

2. MATERIALS AND METHODS

2.1 Microorganism and Strain Maintenance

The fungus used in this work, *Aspergillus clavatus* NRRL1 was kindly provided by Fabio M. Squina from Universidade de Sorocaba, Brazil. This strain was cultivated on PDA (potato dextrose agar) (Sigma-Aldrich®) culture medium previously autoclaved at 1.5 atm for 20 minutes and stored at the Mycology Collection of Laboratório de Microbiologia e Biologia Celular from the Departamento de Biologia at the Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - USP, Ribeirão Preto, São Paulo, Brazil. Samples of the fungus were stored in refrigerator and also kept at – 80 ºC in solution of 20% glycerol and/or at 4 ºC in silica gel, as previously reported [19].

2.2 Time-Course of Xylanase Production by *A. clavatus*

The production of the crude extract containing the xylanase was carried out by the inoculum of one milliliter of 10^7 spores/mL in 50 mL of minimal medium [20] with pH 6.5, at 35 °C, and 1% paper sludge pretreated with HCl [21]. The fungus was incubated for 1 to 14 days in a microbiological incubator at 120 rpm and 70% relative humidity. Each day of the experiment, a different Erlenmeyer was removed from the incubator. As previously described, the extracellular extract was obtained by filtration, and the enzymatic and specific activities were determined with the extracellular extract.

2.3 Effect of the Mixing Conditions and Paper Sludge Concentration on the Secretion of the Xylanase

The fungus was incubated in minimal medium pH 6.5, 35 °C, and 1% paper sludge pretreated with HCl, for 5 days, under static or stirring (120 rpm) conditions. In another experiment, *A. clavatus* was cultivated at the stirring (120 rpm) condition, with pH 6.5, 35° C, for 5 days using different concentrations (1 to 10%) of paper sludge pretreated with HCl. The enzymatic and specific activities were determined with the extracellular extract.

2.4 Effect of Temperature and Initial pH on Xylanase Production by *A. clavatus*

The fungus was incubated in a minimal medium, pH 6.5, with 5% paper sludge pretreated with HCl, for 5 days, under 120 rpm, and different temperature values: 25, 30, 35, and 40 ºC. After that, different pH values: 3.0; 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0, were tested using the same conditions previously mentioned and 35 ºC as cultivation temperature. The enzymatic and specific activities were determined with the extracellular extract.

2.5 Obtaining the Extracellular Crude Extract, Enzymatic Activity, and Protein Determinations

All the culture media were subjected to vacuum filtration using Büchner funnel and Whatman filter paper no 1. The filtrates containing the endo-1,4-β-xylanase were used as an extracellular source for the experiments. Xylanase activity of the extracellular crude extract was determined by the quantification of the released reducing sugars using 3,5-dinitrosalicylic acid (DNS) [22] after incubation with 1% beechwood xylan (Sigma-Aldrich®) in 0.1 M sodium citrate buffer, pH 5.0, at 50 ºC. The absorbance was measured at 540 nm in a Packard SpectraCount™ plate reader, and reducing sugars were quantified using a standard curve with xylose (0.1 to 1.0 mg/mL). The activity unit (U) was defined as the amount of enzyme that generates 1 µmol of reducing sugars/min under the assay conditions.

The Bradford method determined the proteins present in the crude extracellular extracts [23] using bovine serum albumin as standard. The readings were performed in a spectrophotometer

at 595 nm, and the results were expressed in milligram per milliliter. The specific activity was expressed in the total U/total mg protein of the extracellular extract.

2.6 Electrophoretic Profile (SDS-PAGE) of *A. clavatus* Crude Extract Grown in Paper Sludge

After the culture conditions were standardized, the homogeneity and molecular mass of the crude extract containing the endo-1,4-beta-xylanase produced by *A. clavatus* was determined by 12% SDS-PAGE (w/v) pH 8.9. First, the crude extract was concentrated and prepared through dilution in SDS sample buffer in proportion 1:1. Then, electrophoresis was carried out at room temperature in a buffer consisting of 0.025 M Tris-HCl, 0.025 M tricine, and 0.1% SDS (w/v), pH 8.9. The electric flow applied was 40 mA and 120 V. The molecular mass markers used were the Precision Plus Protein™ All Blue Standards (product code #1610373), ranging from 250 to 10 kDa produced by Bio-Rad®.

2.7 Chromatography Analysis of the Hydrolysis Products

Thin-layer chromatography (TLC) was used to identify the XOSs formed after the hydrolysis of the beechwood xylan (Sigma-Aldrich®) by the endo-1,4-β-xylanase. The enzyme was incubated with 1% beechwood xylan (Sigma-Aldrich®) as substrate at 50 ºC for 5; 10; 20; 30, and 60 min. After that, samples were boiled for 5 min and time zero was previously prepared by boiling the enzyme for 5 minutes. The assay mixtures (5 µL) were applied to TLC. The plate was subjected to two sequential ascending chromatography runs using butanol/ethanol/water (5:3:2) as the solvent system and subsequently revealed with 0.2% orcinol-sulfuric acid-methanol (1:9) (w/v). TLC visualization was possible by spraying the mixture on the plate, drying it, and heating at 100°C until the bands of the corresponding xylan hydrolysis products were revealed [24]. A mixture of (1%) xylose, xylobiose, and xylotriose was applied as standard.

2.8 Reproducibility of the Results

All data were independently performed in triplicates and have shown consistent results.

3. RESULTS

3.1 Time-Course of Xylanase Production by *A. clavatus*

A. clavatus kept its high enzymatic activity production in the range between 4 to 14 days of cultivation. The maximum levels of the endo-1,4-beta-xylanase activity were observed between the fifth and tenth day, being around 71.12 ± 1.69 U/mL. This result represented 31% more activity than marked on the fourth day of cultivation (Fig. 1a). Regarding the specific activity, on the fifth day of growth, the extracellular xylanase had 316.12 ± 1.56 U/mg protein. In comparison, on the sixth day, it showed 293.76 ± 2.13 U/mg protein and continued to drop gradually over the days (Fig. 1b). According to these results, 5 days of culture were selected since no significant difference in enzyme activity was observed between 5-10 days, and the best specific activity was observed in 5 days. Also, choosing the fifth day is advantageous since it would have less incubation time for the microorganism, saving resources and production time.

3.2 Effect of the Mixing Conditions on the Secretion of the Xylanase

After 5 days of cultivation, it was observed that *A. clavatus* showed the best xylanase activity in the stirring condition (74.81 ± 2.31) compared to the static condition (10.23 ± 1.36). This activity was 7.31-fold higher than the one observed in the static condition. It was also observed that in the stirring condition, the endo-1,4-beta-xylanase showed a specific activity of 356.24 ± 5.22 U/mg protein. This result represents 1,7-fold more activity than that observed at the static condition. Then, the stirring condition was selected as the ideal for the endo-1,4-beta-xylanase production by *A. clavatus*.

3.3 Effect of the Paper Sludge Concentration on Xylanase Production by *A. clavatus*

The paper sludge concentration was shown to be essential for positively improving endo-1,4-beta-xylanase production. During this experiment, *A. clavatus* increased its enzyme activity conditioned to the rise of paper sludge concentration until 5% paper sludge, reaching the activity of 166.06 ± 7.63 U/mL. This result represented 92% increase of the activity produced with 1% of paper sludge (Fig. 2a). Furthermore, the specific activity of the enzyme

also reached its highest value (638.79 ± 11.06 U/mg protein) with 5% paper sludge. This specific activity is approximately 21 and 25% above the specific activity observed with 4 and 6% paper sludge (Fig. 2b).

3.4 Effect of Temperature on Xylanase Production by *A. clavatus*

A. clavatus cultivation at 35ºC showed 184.29 ± 6.12 U/mL of activity, representing the higher endo-1,4-beta-xylanase activity observed. This activity was 6-fold higher than that observed at 25ºC and 32% higher than that produced at 30ºC. During the incubation at 40ºC, *A. clavatus* showed no endo-1,4-beta-xylanase activity (Fig. 3a). At 35°C, the endo-1,4-beta-xylanase also reached the highest specific activity, showing 658.18 ± 16.12 U/mg protein (Fig. 3b). Based on these results, 35ºC was selected for the production of the endo-1,4-beta-xylanase by *A. clavatus*.

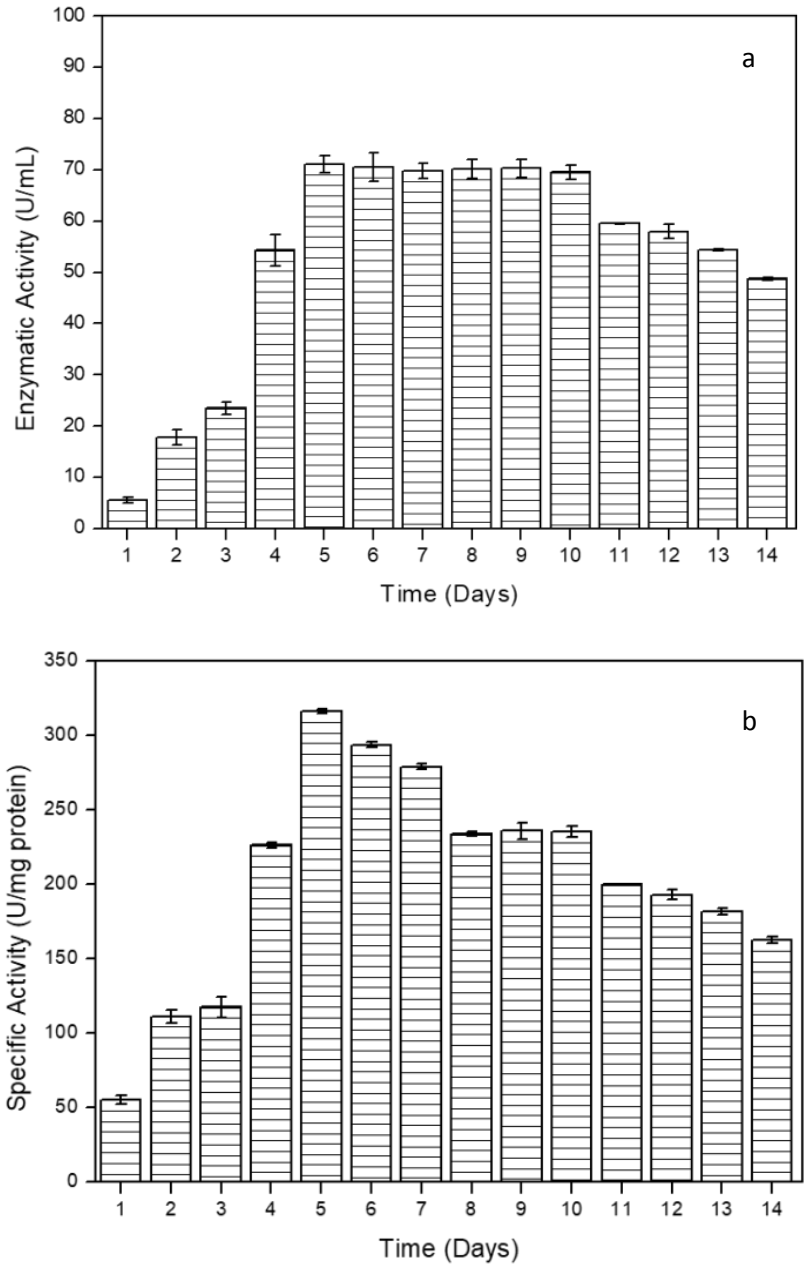

Fig. 1. Time-course of the production (a) and specific activity (b) of the endo-1,4-beta-xylanase from *A. clavatus*. The fungus was incubated in a minimal medium with 1% paper sludge as a carbon source at 35 ºC, pH 6.5 and 120 rpm

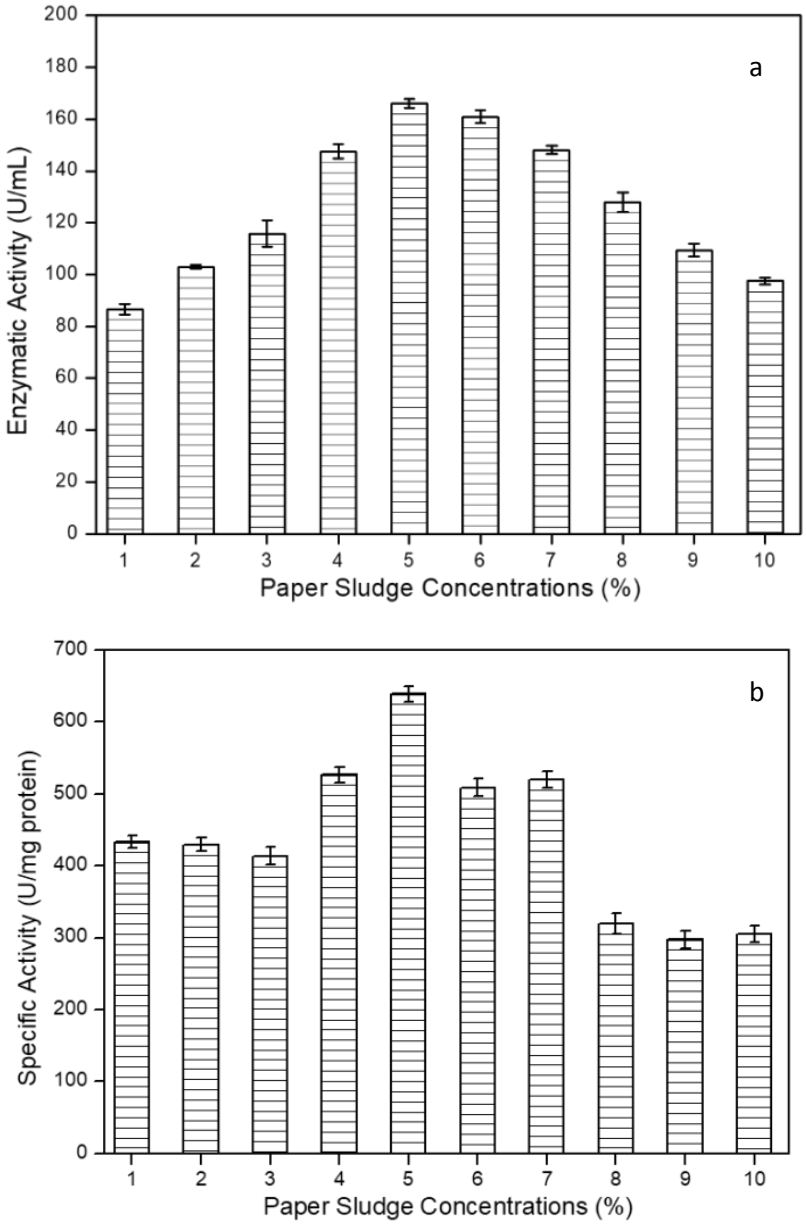

Fig. 2. Effect of different paper sludge concentrations in the production (a) and specific activity (b) of the endo-1,4-beta-xylanase from *A. clavatus*. The fungus was incubated in a minimum medium, at 35 ºC, pH 6.5, for 5 days, under 120 rpm

3.5 Effect of the Initial pH on Xylanase Production by *A. clavatus*

The best initial pH value of the medium for the enzyme production was 6.0, in which the activity reached 191.54 ± 4.38 U/mL (Fig. 4a). In the pH range of 3.0-5.0, the enzyme production increased conditioned to the rose in pH values. In the cultures with initial pH ranging from 5.5 to 6.5, *A. clavatus* maintained high enzyme production with relative activity of 78.3% at pH 5.5 and 83.4% at pH 6.5. At pH 7.0 and 7.5, a significant decrease in the activity was observed, but it still showed 40% and 19% of the relative activity, respectively, compared to pH 6.0. Finally, at pH 8.0, the enzyme demonstrated relative activity of 10.2%. Furthermore, it was possible to observe that, at pH 6.0, the specific activity of the xylanase was higher (684.09 ± 5.57 U/mg protein) than the other initial pH values tested (Fig. 4b). These results suggest higher tolerance at different pH values for the enzyme produced by *A. clavatus.*

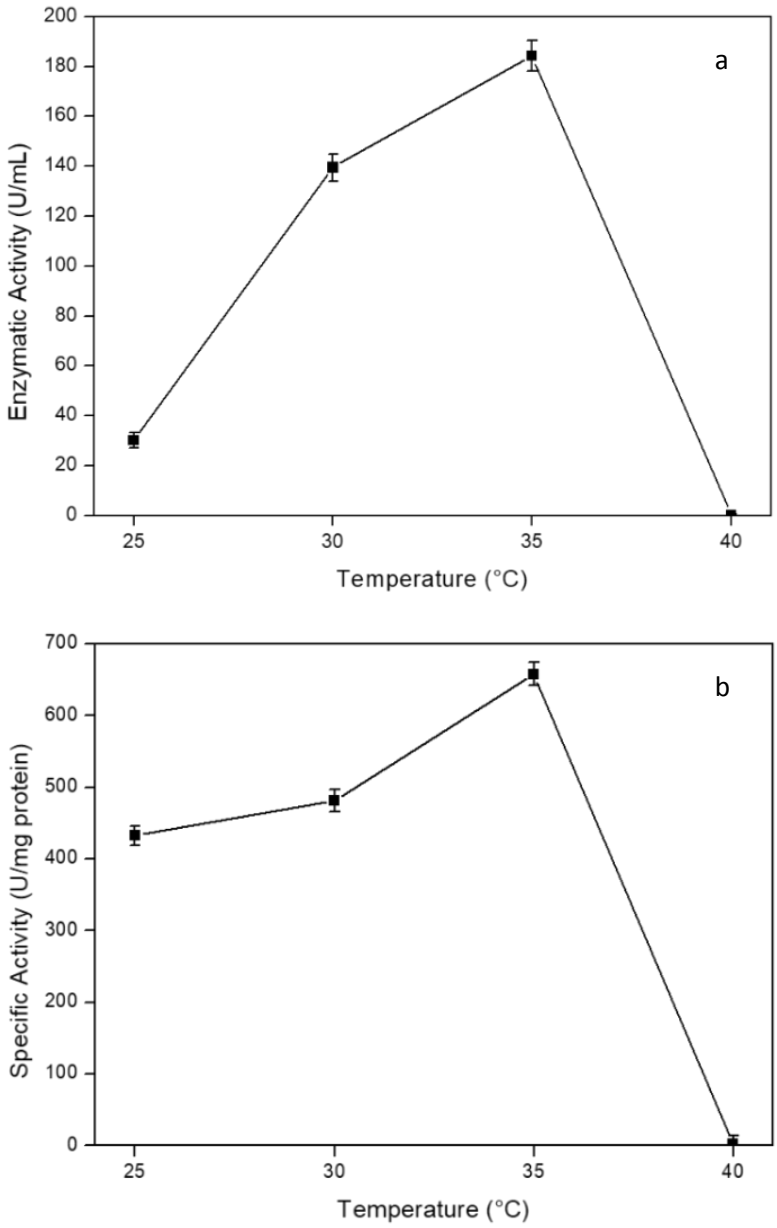

Fig. 3. Effect of temperature in the production (a) and specific activity (b) of the endo-1,4-beta-xylanase from *A. clavatus*. The fungus was incubated in minimal medium with 5% paper sludge as carbon source, pH 6.5, for 5 days, under 120 rpm

3.6 Electrophoretic Profile (SDS-PAGE) of *A. clavatus* Crude Extract Grown in Paper Sludge

After the standardization of the culture conditions, *A. clavatus* was incubated in minimal medium with 5% paper sludge as carbon source, at 35 ºC, for 5 days, under 120 rpm, and the crude extract produced was subjected to SDS-PAGE analysis. After revealing the gel with Coomassie Brilliant Blue, it was possible to observe a few bands of proteins with molecular masses of 25 (black arrow), 44 (red arrow), 55 (blue arrow), 67 (orange arrow), and 80 kDa (green arrow) (Fig. 5). This result was already expected according to the screening results that previously revealed the presence of ß-glucosidase and ß-xylosidase in low concentrations [19]. However, the band corresponding to the endo-1,4-beta-xylanase was the most intense in the gel (Fig. 5 - black arrow), which justified the high activity demonstrated in the enzymatic experiments compared with the other proteins observed. Also,

it was possible to infer that this band represents the endo-1,4-beta-xylanase due to its molecular mass of 25 kDa, a molecular mass commonly detected for xylanases of fungal origin and previously reported by Pasin et al. [7]. It is essential to mention that this xylanase from *A. clavatus* had been previously predicted by genomic studies [25]. Therefore, it was proposed that this enzyme would have precisely the same characteristics observed here. Then, it is possible to infer that the xylanase from *A. clavatus* is part of the GH11 family, as previously proposed by Segato et al. [25]. This result confirmed the high importance of studying this enzyme. Furthermore, it is a predicted enzyme that is being high produced, making later experiments less expensive and interesting for biotechnological application.

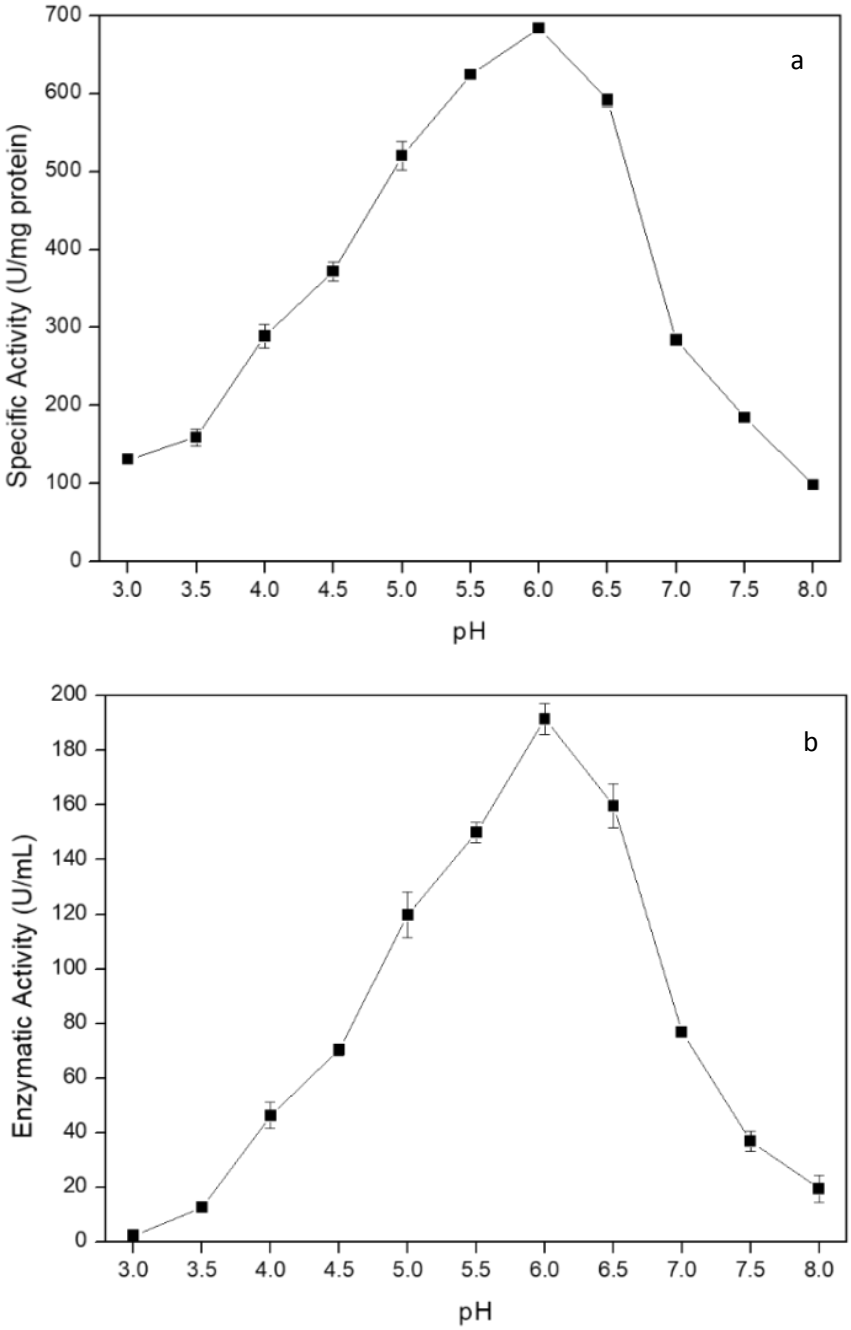

Fig. 4. Effect of pH in the production (a) and specific activity (b) of the endo-1,4-beta-xylanase from *A. clavatus*. The fungus was incubated in minimal medium with 5% paper sludge as carbon source, at 35 ºC, for 5 days, under 120 rpm

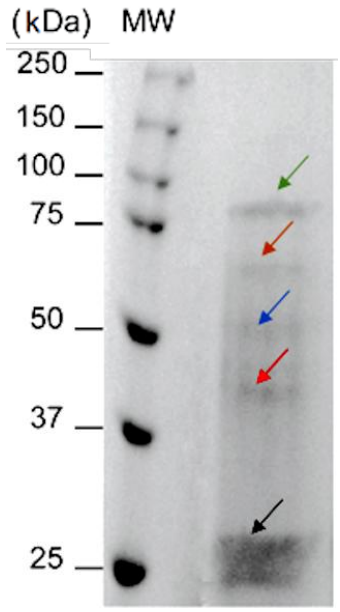

Fig. 5. Electrophoresis analysis of the crude extract containing the endo-1,4-beta-xylanase produced by *A. clavatus* in paper sludge. The black, red, blue, orange and green arrows indicate the bands of 25, 44, 55, 67, and 80 kDa, respectively

3.7 Analysis of the Products from the Hydrolysis and Xylooligosaccharides production by TLC

The endo-1,4-beta-xylanase showed the formation of different XOSs when incubated with 1% beechwood xylan (Sigma-Aldrich®) as substrate, in the assay times of 5; 10; 20; 30, and 60 minutes. In these conditions (5-60 min), the enzyme could hydrolyze the substrate to xylobiose, xylotriose, and xylotetraose (Fig. 6). These results confirmed the biotechnological potential of this enzyme to be applied in industrial XOS production using different assay times.

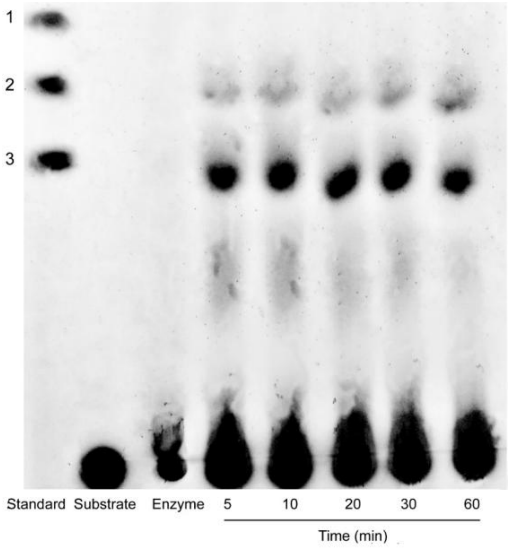

Fig. 6. Hydrolysis profile of the endo-1,4-beta-xylanase produced by *A. clavatus* when incubated with 1% beechwood xylan at 5; 10; 20; 30; 60 minutes. As standard sugars, xylose, xylobiose, and xylotriose were used. These substrates are represented by the numbers 1, 2, and 3, respectively. Blanks were previously established by the individual boil of the substrate and enzyme for 5 minutes, followed by application in the TLC

4. DISCUSSION

The fungus *A. clavatus* is already known in the scientific literature as a good producer of several commercially and interesting enzymes, such as proteases [26], feruloyl esterases [27], and cellulases [19]. However, there are no reports in the literature on standardizing and obtaining high levels of endo-1,4-beta-xylanase (191.54 ± 4.38 U/mL) produced by *A. clavatus* when grown in HCl-treated paper sludge, as observed in this work. Thus, this work represents unprecedented data for the scientific community since it has not been reported before.

Besides that, it obtained high levels of endo-1,4-beta-xylanase (191.54 ± 4.38 U/mL) produced by *A. clavatus* when cultivated in paper sludge. There are many reports in the literature of xylanases produced with lower levels of activity when compared to those observed here. However, these enzymes were produced using different residues and fungi from those used in this work. For example, it is possible to find in the literature xylanases from *Aspergillus flavus* (80 U/mL) [28], *Thermoascus aurantiacus* strain SL16W (19 U/mL) [29], and *Fusarium oxysporum* (7.92 U/mL) [30] produced with rice straw, red tea leaves and corncob powder, respectively. It is important to mention that the paper sludge used in this work represents an agro-industrial waste that causes several environmental damages due to its uncontrolled disposal, which reinforces the importance of the present study. The enzyme produced by *A. clavatus* has shown high concentrations in its crude extract, as observed in the results. This observation suggests the potential of extensive use of this enzyme in industrial applications since its high concentration decreases the necessity of subsequent processes, such as large-scale separation, concentration, and purification from the raw extract. This kind of approach can represent the most expensive part of biomolecules production [31]. Different cultivation conditions of *A. clavatus* were evaluated to make this enzyme more feasible, and the best enzyme production was observed with five days of culture. It can be assumed that during this time, the fungus absorbed all the easily assimilated nutrients from the culture medium and reached sufficient maturity to secrete large concentrations of complex enzymes, such as xylanases, in order to degrade and absorb complex nutrients from the culture medium. This result is similar to that obtained by Chen et al. [32], who improved xylanase production by *Aspergillus flavus* after 5 days of cultivation. The same result was also observed by Menezes et al. [33], who obtained the best xylanase activity by *Aspergillus brasiliensis* BLf1 and recombinant *Aspergillus nidulans* XynC A773 after 5 days of culture.

The best mixing condition for the xylanase production was observed using 120 rpm, according to Miao et al. [34] that obtained the maximal xylanase activity by *Aspergillus fumigatus* Z5 when incubated at 150 rpm. The higher xylanase production by fungi at mixing conditions can be explained by the high aeration of the culture and the intimate contact between the fungal mycelium and the paper sludge since the attachment of the fungus to the paper sludge surface is increased with the mixing conditions [35].

Amorim et al. [36] studied a *Trichoderma reesei* strain that showed the maximal endo-xylanase activity (39.7 ± 4.1 U/mL) after 5 days of fermentation with 20 g/L (2%) of brewer's spent grain. At higher concentrations, inhibition of the enzyme production was observed. Sinjaroonsak et al. [37] also verified the same effect for *Streptomyces thermocoprophilus* strain TC13W that showed the highest cellulase (6.5 U/ml) and xylanase (9.9 U/ml) activity in a medium with 5.0 g/L (0.5%) of tuna condensate powder, presenting inhibition in the production of the enzymes with higher concentrations of this substrate. According to the literature, sometimes the inhibition on the enzyme production may be caused by the substrate, but also by mass transfer and aeration issues, possibly because of the increase in viscosity and density of the reaction mixture when higher concentrations of the substrate are present [36,38]. However, this phenomenon was not observed in this work. Here, the best endo-1,4-beta-xylanase activity was obtained using 5% of paper sludge as substrate, and during the increase of paper sludge concentrations, the enzyme activity maintained almost the same. With this result, it can be assumed that the paper sludge and its hydrolysis products have no inhibition effect on enzyme production. This is a particularly important result for large-scale industrial production of the xylanase from *A. clavatus* using paper sludge as substrate.

At 35 ºC, *A. clavatus* reached the best enzyme production. This result was already expected since *A. clavatus* is a well-known mesophilic species [39]. Therefore, its optimal temperature for growth and enzyme production should vary

between 25-35 ºC. Nevertheless, it is a different result from that observed by Monclaro et al. [40], who obtained the best xylanase production by *Aspergillus tamarii* at 28 ºC. On the other hand, Chen et al. [41] observed similar results with endo-1,4-beta-xylanase that showed its best production when cultivating *Aspergillus flavus* at 35 ºC.

Cunha et al. [42] obtained the highest levels of xylanase production by *Aspergillus foetidus* in soybean residue using initial pH of 7.0. However, Dos Santos et al. [43] studied the fungus *Aspergillus* cf. *tubingensis,* which showed the best xylanase production at the initial pH of 6.0. This result is in concordance with the one obtained in this work, where the best initial pH was 6.0. It is already widely known that fungi naturally prefer more acidic environments [44]. So, this result is in accordance with state of the art so far. It can be assumed that the preference for acidic pH is related to an environment with less competition between fungi and bacteria, in addition, the acidic environment favors the fungus by facilitating the enzyme activity on the substrate, as the optimal pH of the majority of enzymes is between 5-7 [8].

Nowadays, xylobiose and xylotriose are the most common XOSs produced by the enzymatic hydrolysis of endo-xylanases [32,45]. However, the endo-1,4-beta-xylanase produced by *A. clavatus* exhibited a little different pattern of hydrolysis. It was able to break the beechwood xylan and yielded significant amounts of xylobiose, xylotriose, and xylotetraose. Also, a slight decrease in the concentration of xilotetraose and xylotriose was observed concomitant with the increase in xylobiose during the assay time. This result could be explained by the previous presence of ß-glucosidase and ß-xylosidase in low concentrations [18].

Furthermore, several ß-xylosidases described in the literature could hydrolyze xylotetraose and xylotriose and release xylobiose [46,47]. Also, it is possible that the ß-glucosidase showed some hydrolysis activity on xylotetraose and xylotriose, as previously observed by Zhou et al. [48]. The xylobiose and xylotriose have gained much attention because of their use as a higher-value food supplement (prebiotic), promoting the proliferation of Bifidobacteria, a beneficial microorganism for human intestines and which reduces the risk of colon cancer [32,49].

5. CONCLUSION

In conclusion, the new and highly active endo-1,4-beta-xylanase from *Aspergillus clavatus* was produced using paper sludge, one of the significant wastes generated by the paper and pulp industries in the world. The enzyme had its molecular mass determined as 25 kDa and showed to be produced at high concentrations by the fungus. Furthermore, the culture conditions, such as time-course (5 days), mixing state (agitation), paper sludge concentration (5%), pH (6.0), and temperature (35 ºC), were optimized and its production was significantly improved. Furthermore, it was observed that the endo-1,4-beta-xylanase hydrolyzed beechwood xylan up to pronounced amounts of xylobiose, xylotriose, and xylotetraose in short periods, which makes this enzyme with interesting characteristics when compared to the other endo-1,4-beta-xylanases already described. These features bring to light the potential for biotechnology application of this enzyme to produce high XOS concentrations under adverse conditions.

CONSENT

No animals or humans were used in this study.

ETHICAL APPROVAL

No animals or humans were used in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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