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PRODUCTION OF XYLANASE USING SUGARCANE INDUSTRY RESIDUE TO OBTAIN XYLO-OLIGOSACCHARIDES

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ABSTRACT

The use of agro-industrial residue in new processes has encouraged the development of biotechnological strategies for obtaining biomolecules with various potential applications. Sugarcane bagasse and straw—the primary byproducts of the sugarcane industry—are rich in hemicellulose, serving as an alternative and low-cost source for obtaining enzymes and prebiotics. In this context, this study aims to investigate the production of xylanases through solid-state fermentation using different microorganisms, substrates, and process conditions. *Aspergillus niger* CCT 4355 and *Trichoderma reesei* CMAA 1168 were cultivated on sugarcane bagasse and straw under different moisture conditions (53%, 69%, and 76%). The produced xylanases were characterized concerning their optimal pH (2.5–8.0) and temperature (20–80 °C). The highest enzymatic activity was obtained from *T. reesei* CMAA 1168 cultivated on sugarcane straw (6.99 U/mL) with 76% moisture, which was 17% higher than that produced from *A. niger* CCT 4355. Additionally, this enzyme exhibited a slightly more acidic optimal pH range (3.0–5.0) compared to that produced from *A. niger* CCT 4355, as well as greater thermal stability (45–55 °C).

Keywords: *Aspergillus niger* CCT 4355, *Trichoderma reesei* CMAA 1168, agro-industrial residue, xylanases.

1. INTRODUCTION

The use of agro-industrial residue has attracted great interest in various industrial sectors since these can be transformed into high-value biomolecules [1]. Specifically, the residue from the sugarcane industry, such as sugarcane bagasse and straw, represents an abundant and promising source for producing enzymes, such as xylanases. Brazil, the world's largest producer of sugarcane, generated 711.2 million tons in the 2023/2024 harvest [2] and, of this amount, approximately 91 million tons are bagasse [3] and 100 million tons are straw [4]. Sugarcane residues have a composition rich in hemicellulose, especially xylan, making them ideal substrates for enzyme production [5,6]. These enzymes, derived from the cultivation of filamentous fungi on solid substrates, have the potential to hydrolyze xylan [7], constituting an essential raw material for the production of prebiotics and other industrial applications [8]. Solid-state cultivation with filamentous fungi offers advantages such as low substrate cost, reduced water and energy consumption, lower risk of contamination, and high productivity [9], in addition to being optimizable for the efficient production of commercially relevant biomolecules, including xylanases.

2. MATERIALS AND METHODS

The fungi *A. niger* CCT 4355 and *T. reesei* CMAA 1168 were used for xylanase production using different residues from the sugarcane industry in cultures with varying moisture levels. The cultures were conducted in 125 mL conical flasks, each containing 5 g of sugarcane bagasse or straw, with different moisture levels (53, 69, and 76%) achieved by adding basal medium (pH 5.5)[10]. The inoculum was prepared by suspending spores of the different strains at a concentration of 10^6 spores per gram of substrate. The flasks were kept in a BOD incubator at 30 ºC for 8 days and samples were collected periodically every 48 hours. The extraction of the enzyme extract was performed in a rotational incubator (30 ºC, 150 rpm for 30 min) by adding 50 mL of sodium acetate buffer (50 mM, pH 5.0) to the flasks, followed by centrifugation (4 ºC, 3000 × g for 15 min). The optimal pH of the enzymes obtained from the different fungi was evaluated using different buffers (50 mM) at various pH levels (2.5–8.0), maintaining a constant temperature (50 ºC). The optimal temperature was assessed under different conditions (20–80 ºC) using 50 mM acetate buffer (pH 5.0). Xylanase enzyme activity was measured using a reaction mixture containing 500 µL of 1% xylan solution (50 mM sodium acetate buffer, pH 5.0) and 500 µL of crude enzyme extract (at 50 ºC for 30 min). The reaction was stopped by adding 1 mL of DNS solution in a thermostatic bath at 100 ºC for 5 min. The samples were then analyzed using a spectrophotometer with readings at a wavelength of 540 nm. The concentration of reducing sugars was determined using xylose as the standard. One unit of xylanase activity is defined as the amount of enzyme that releases 1 µmol of xylose per minute under the assay conditions.

3. RESULTS AND DISCUSSION

The experiment was conducted to evaluate the influence of moisture on the xylanase enzyme production profile by the fungi *A. niger* CCT 4355 and *T. reesei* CMAA 1168. As shown in Figure 1, the highest enzymatic activity was obtained at the highest moisture level (76%) for both fungi and substrates tested. For the cultivation of *A. niger* CCT 4355 (Figure 1 A) in sugarcane bagasse, the highest enzyme production occurred at 96 hours (5.82 U/mL) with 76% moisture. For moisture levels of 69 and 53%, the enzymatic activities were 9% (5.32 U/mL) and 29% (4.12 U/mL) lower than the maximum, respectively. When *A. niger* CCT 4355 was cultivated in sugarcane straw, its maximum enzymatic activity (5.47 U/mL) was similar to that obtained in bagasse, but a longer cultivation time was required (192 h), which consequently reduced the process productivity.

Regarding the xylanase produced from *T. reesei* CMAA 1168 (Figure 1B) in sugarcane bagasse, it was quite similar to that obtained from *A. niger* CCT 4355 (5.47 U/mL). However, the moisture effect on *T. reesei* CMAA 1168 was more pronounced, with a 73% decrease in maximum enzymatic activity (1.47 U/mL) observed at 53% moisture, and 30% lower (3.84 U/mL) at 69% moisture. In sugarcane straw, the highest enzymatic activity values (6.99 U/mL) were obtained at 76% moisture, which was 17% higher than the maximum obtained from *A. niger* CCT 4355. This indicates that this fungus is influenced by the type of substrate where it is cultivated. Since this was the best condition for obtaining xylanase, this strain will be used for future process optimization steps.

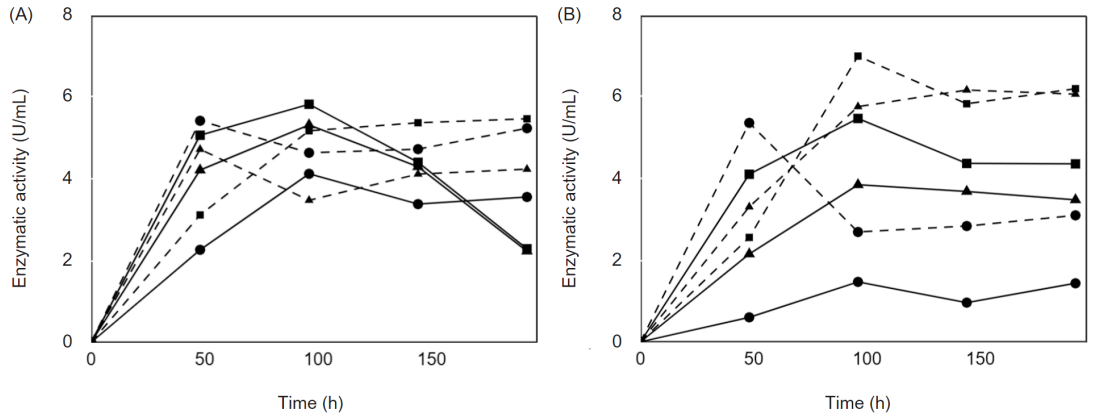

Figure 1. Enzymatic activity of the crude enzymatic extract of xylanases from (A) *A. niger* CCT 4355 and (B) *T. reesei* CMAA 1168 obtained in culture at 30 ºC for 192 hours in sugarcane (—) bagasse and (---) straw at different moisture levels: (●) 53%, (▲) 69%, and (■) 76%.

Figure 2 presents the optimal pH and temperature of the enzymes produced from the two fungi. For the enzyme from *A. niger* CCT 4355, the pH range remained stable from 3.0 to 6.0, with relative activities above 86%, and an optimal pH of 5.0. The optimal temperature was 50 ºC, with the optimal range between 45 ºC and 55 ºC, with relative activities of 87% and 82%, respectively.

For the enzyme from *T. reesei* CMAA 1168, a relatively more acidic pH range (3.0–5.0) was observed compared to that obtained from *A. niger* CCT 4355, with optimal pH achieved at 3.5 and relative activities ranging from 98% to 90%, respectively. Regarding the optimal temperature, it was obtained at 50 ºC, with the optimal temperature range between 45 ºC and 55 ºC, with relative activities of 99% and 92%, respectively.

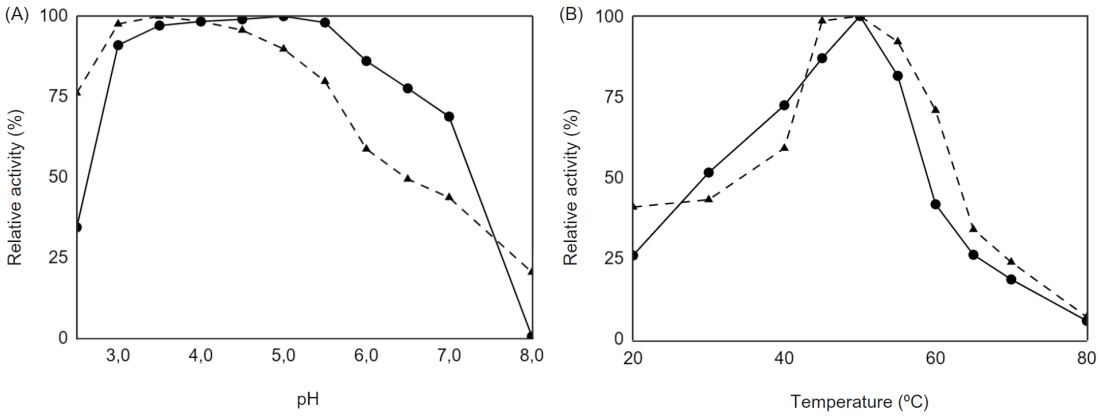

Figure 2. Optimal activities for (A) pH and (B) temperature of the crude enzymatic extracts of xylanase obtained from (●) *A. niger* CCT 4355 and (▲) *T. reesei* CMAA 1168.

4. CONCLUSION

The solid residues from the sugarcane industry demonstrated biotechnological potential for obtaining xylanases, particularly sugarcane straw when associated with the fungus *Trichoderma reesei* CMAA 1168. This contributes to a more sustainable production of biomolecules with significant global commercial importance and high biotechnological interest in the food, pharmaceutical, and agricultural industries.

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