AUTOHYDROLYSIS PRETREATMENT OF CORNCOB FOR CELLULASE PRODUCTION BY *TRICHODERMA REESEI* MUM 97.53

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ABSTRACT – Microbial cellulase production has attracted great attention due its several applications. In this context, studies that become this practice feasible are very important. Corncob is an inexpensive byproduct, which contains more than 30% of cellulose; however, this material is not readily available to enzymatic hydrolysis and pretreatment of lignocellulosic material in autohydrolysis processes become this more easy. The inclusion of this pretreated material in the nutrient media can be a strategy to increase and undervalue cellulase production. The best pretreatment conditions for total cellulase (FPase), β-glucosidase and exoglucanase (avicelase) were obtained using the solid fraction pretreated at 200ºC for 30 or 50 minutes. It was not detected endoglucanase production (CMCase) using corncob without treatment. However, its induction was observed when *T. reesei* was cultivated with the corncob liquid fraction obtained after 30 minutes of pretreatment, mainly at 200ºC, where the production was maximal.

1. INTRODUCTION

Lignocellulosic materials are mainly made up of cellulose, hemicelluloses and lignin. However, the cellulose in the plant cell wall is not readily available to enzymatic hydrolysis (cellulases) mainly due to the low accessibility of microcrystalline cellulose fibers and the presence of lignin (mainly) and hemicellulose on the surface of cellulose, which prevents cellulases to act efficiently. Thus, pretreatment of lignocellulosic residues is a prerequisite and it can be performed by different methods (Dashtban et al. 2009).

Recently, autohydrolysis method has gained interest, as more environmental-friendly technologies (Garrote et al. 1999). It has been mainly used as a pretreatment to make cellulose more amenable to further enzymatic saccharification (Carvalheiro et al. 2004); but can also be as promising technology for converting agroindustrial residues into useful substrate for fermentation, since no chemicals other than water are used.

In this work, pretreated corncob has been used as substrate to cellulase production. Microbial cellulases have become the focal biocatalysts due to their complex nature and wide spread industrial applications, as biomass refining, pulp and paper, textile, food, and animal

feed. It is a family of at least 3 groups of enzymes: endo-(1,4)-β-D-glucanase (EC 3.2.1.4) exo-(1,4)-β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21). The exoglucanase (CBH) acts on the ends of the cellulose chain and releases β-cellobiose as the end product; endoglucanase (EG) randomly attacks the internal *O*-glycosidic bonds, resulting in glucan chains of different lengths; and the β-glucosidases act specifically on the β-cellobiose disaccharides and produce glucose (Kuhad et al. 2011). These enzymes have been commercially available for more than 30 years, and have represented a target for both academic as well as industrial research.

2. MATERIALS AND METHODS

Corncob was kindly supplied by a local farmer (Caide, Lousada, Portugal). The material was dried at 40 ºC in an oven for 12 h, and after it was cut into small pieces (1 cm) and milled in a knives mill to pass through a 1.0 mm.

Corncob samples and water were mixed in a closed and pressurized vessel in order to obtain a solid/liquid ratio of 1:10 w/v. The system was heated at 180, 190 or 200 ºC during 10, 30 or 50 min. The liquid phase or liquor (hemicelluloses rich fraction) was separated from the solids (cellulose fraction rich) by filtration and used as substrate for fermentations.

The microorganism used in this work was the fungal strain *Trichoderma reesei* MUM 97.53. It was kindly provided by Dr. Nelson Lima from MUM – Micoteca da Universidade do Minho (Portugal). This fungus was maintained at 30 °C, on slants of solid PDA media.

Conidia from 7-day-old cultures, with cell concentration of 1×10^{9} cells.mL^{-1}, were inoculated into 250 mL Erlenmeyer flasks containing 50 mL of the liquid medium described by Mandels (1969), pH 6.0, containing the carbon source: 1% (w/v) corncob solid fraction; 100% (v/v) corncob autohydrolysis liquor; or combination of 1% (w/v) corncob fraction solid and 10% (v/v) corncob autohydrolysis liquor. One per cent of corncob without treatment was used as control. The cultures were incubated at 30 °C, for 100 rpm, for six days. After, the extracellular extract was filtrated, and used as source of extracellular enzymes.

Cellulases activities were determined by DNS, using glucose as standard (Miller, 1959). Endoglucanase activity was detected using 1% (w/v) carboxymethyl cellulose in 100 mM sodium acetate buffer, pH 4.0, as substrate. Reaction mixture containing 0.2 mL enzyme and 0.2 mL substrate was incubated at 55ºC for 30 minutes. Exoglucanase activity was detected using 1% (w/v) avicel in 100 mM sodium acetate buffer, pH 5.0, as substrate. Reaction mixture containing 0.2 mL enzyme and 0.2 mL substrate was incubated at 55ºC for 1 hour. Total cellulase was detected using filter paper, as substrate. Reaction mixtures contained 1 strip of Whatman grade nº 1 filter paper (10 x 30 mm), 0.5 mL of 100 mM sodium acetate buffer, pH 5.0, and 0.5 mL of enzyme. The mixture was incubated at 55ºC for 1 hour.

β-glucosidase activity was assayed using 5 mM *p*-nitrophenyl-β-D-glucopyranoside (PNP-glu) as substrate. Reaction mixtures containing 0.1 mL enzyme, 0.1 mL of 50 mM sodium citrate buffer, pH 4.5, and 0.3 mL substrate in distilled water. The mixture was incubated at 50ºC for 10 minutes. The released *p*-nitrophenolate was estimated with saturated sodium tetraborate solution, using *p*-nitrophenol as standard (Kersters-Hilderson et al., 1982).

One unit of cellulase was defined as the amount of enzyme that released 1 μmol of product per minute under the conditions of the assay.

3. RESULTS AND DISCUSSION

According to Figure 1, the best condition for cellulase production was observed when the microorganism was mainly cultivated with 1% (w/v) of the solid fraction obtained after the autohydrolysis pretreatment at 200ºC.

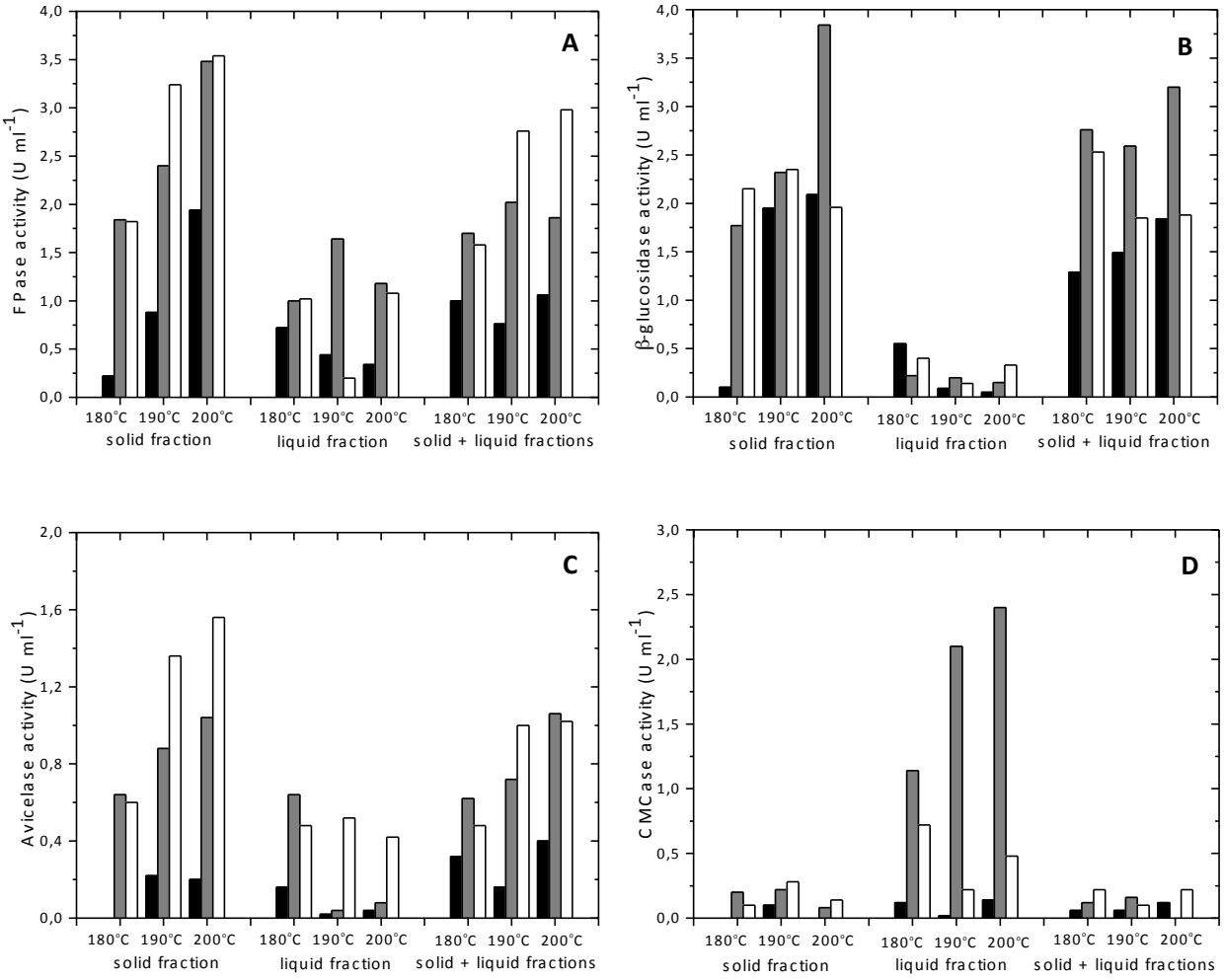

Figure 1. Performance of *Trichoderma reesei* during fermentation in Mandels medium for total cellulase or FPase (A), β-glucosidase (B), exoglucanase or avicelase (C) and endoglucanase or CMCase (D) productions using different fraction of pretreated corncob. Legend: black column – 10 minutes of autohydrolysis; gray column – 30 minutes of autohydrolysis; white column – 50 minutes of autohydrolysis. The microorganism was cultivated at 30ºC, 100 rpm for 6 days.

The total cellulase production (Figure 1A), estimated with paper filter as substrate, was 25% more when corncob was pretreated at 200ºC for 30 or 50 minutes in relation to that obtained with corncob without treatment (2.8 U mL^{-1}). In relation to β-glucosidase activity (Figure 1B), the pretreatment at 200ºC for 30 minutes increased the activity in 92% in relation to corncob without treatment (2 U mL^{-1}), when the solid fraction was used as substrate; and the exoglucanase activity (Figure 1C) obtained with the fraction solid of corncob treated at 200ºC for 50 minutes was 70% higher than corncob without treatment (0.9 U mL^{-1}). Interestingly, no endoglucanase activity was observed with corncob without treatment was used as substrate. However in Figure 1D can be observed a induction of endoglucanase activity using the liquid fraction obtained after the pretreatment at 180, 190 and 200ºC for 30 minutes. The maximal activity was observed in this last (2.4 U mL^{-1}).

4. CONCLUSION

The use of pretreated corncob as substrate for production of cellulolytic enzymes was very promising, since the use of substrate improved the enzymatic production.

5. REFERENCES

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