VALORIZATION OF LIGNOCELLULOSIC INDUSTRIAL RESIDUES FOR XYLANASE PRODUCTION BY AN ANTARCTIC YEAST

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ABSTRACT

Eucalyptus pinchips, a residual product from pulp industries, presents an opportunity as a raw material for the production of high-value bioproducts such as enzymes. Among these enzymes, xylanases hold significant potential for applications in the food, agro-fiber and paper and pulp industries. A prevalent need within these industries aims to reduce process temperatures, prompting an ongoing search for novel microorganisms capable of secreting cold-active xylanases. In this study, we investigated the ability of an Antarctic isolated yeast, *Trichosporon pullulans*, to grow and produce xylanases from xylose and xylo-oligomers rich hemicellulosic hydrolysates. Autohydrolysis, an eco-friendly pretreatment, was applied to pinchips to obtain the hemicellulosic hydrolysate, subsequently used as a cost-effective carbon source for xylanase production. Two detoxification resin methods (XAD-4 and WA-30) were assessed to remove potential inhibitors for microorganisms (organic acid, soluble lignin, phenolic compounds, furfural and HMF). Additionally, two nitrogen sources: corn steep liquor (CSL) and yeast extract (YE), were studied for media supplementation. *T. pullulans* is an interesting candidate for xylanase production for applications that require high enzyme activity (7.6 ± 0.4 IU/mL) at low temperatures when cultivated in non-detoxified hemicellulosic hydrolysates supplemented with YE.

Keywords: Xylanase. Industrial Forestry residue. Autohydrolysis pretreatment. Cold-active enzyme. Psychrotolerant yeast.

1 INTRODUCTION

Lignocellulosic residues, derived from forestry and industrial sector in Uruguay, are renewable resources for the generation of value-added products and energy. Uruguay produces 4.7 million ADT (air dry tonnes) per year of Bleached Eucalyptus Kraft Pulp from a mixture of *E. grandis*, *E. dunnii* and *E. globulus* species. During the chipping process in the pulp mill, 1-2% of the input eucalyptus wood is discarded as pinchips and fines[1,2].

Microorganisms and their carbohydrate-active enzymes (CAZymes) are central for depolymerizing these complex lignocellulosic polysaccharides. Xylan, the major hemicellulose polymer, is hydrolyzed by the concerted action of CAZymes. Xylanases find diverse biotechnological applications, including the bioconversion of lignocellulose into fermentable sugars, clarification of juices and pulp bleaching[3]. However, for industrial applications, emphasis is placed on optimizing xylanases characteristics and reducing production costs.

Psychrophiles and psychrotolerant microorganisms can produce efficient biomolecules such as cold-active enzymes (CAEs), that have shown significant biocatalytic activity at low and moderate temperatures as compared to their mesophilic or thermophilic counterparts[4]. Hydrolases sourced from psychrophilic and psychrotolerant microorganisms, including cellulases and xylanases, are the preferred CAEs, not only for their biotechnological applications, but also for their relevance in the global market of industrial enzymes[5].

Agroresidues and organic wastes (wheat bran, rice straw, sugarcane bagasse, coconut coir, sorghum straw, wood pulp, sawdust, molasses, sugar beet pulp fruit) are used as carbon sources for xylanase production[6]. Despite ongoing efforts, yeast-mediated production of xylanases remains non-competitive. This study explored the feasibility of utilizing an economical lignocellulosic feedstock as carbon and energy source, to enhance the cost-effectiveness of biotechnological xylanase production. The focus of this work was on the valorization of industrial waste (pinchips) for xylanase production using both detoxified and non-detoxified hemicellulosic hydrolysate media culture, utilizing an Antarctic yeast.

2 MATERIAL & METHODS

RAW MATERIAL

Eucalyptus sawdust was supplied by a local pulp mill (UPM-Fray Bentos, Uruguay). The material was dried until 8% moisture content and stored at room temperature.

PRETREATMENT

Thermal treatment was performed in 300 mL rotating cylindrical reactors (Fibretec Inc., India) immersed in a thermostatic bath. An autohydrolysis pretreatment was applied to eucalyptus pinchips, with a P factor of 400, temperature of 165°C and a liquid to solid ratio of 7 g/g dry solid, to yield the hemicellulosic hydrolysate. The pH of liquid fraction post-autohydrolysis was 3.5.

DETOXIFICATION OF EUCALYPTUS HEMICELLULOSIC HYDROLYSATE

The hemicellulosic hydrolysate (HH) obtained was detoxified using exchangeable resins in batch mode, for 30 minutes with a gentle agitation, and with a resin liquid relation of 1:3. The resins studied were: Amberlite® XAD-7 and Diadion® WA-30. Amberlite® XAD-7, is an adsorptive, non-polar polymeric resin that could remove organic inhibitors such as acid soluble lignin from lignocellulosic hydrolysates[7]. Diadion® WA-30, is a weakly basic anion-exchange resin, designed to remove short organic acids in a sugar syrup media. The characterization of the hemicellulosic hydrolysate obtained before and after detoxification was done according to the standard protocol utilized by the National Renewable Energy Laboratory (NREL)[8].

MICROORGANISM

T. pullulans, a psychrotolerant yeast isolated from Fildes Peninsula in King George Islands, Antarctica, was used for xylanase production from hydrolysates. *T. pullulans* is reported as a strain capable of degrading polysaccharides (xylan, cellulose)[9] and a natural producer of hemicellulases. The inoculum was developed by growing the yeast in 500 mL Erlenmeyer flasks with 100 mL of media, which contained in g/L: 20 glucose, 20 peptone, 5 YE, and incubated in an orbital shaker at 150 rpm for 12 h.

ENZYME PRODUCTION

The autohydrolysis pretreated hydrolysate, rich in xylose and xylo-oligomers, was used to study the effect of hemicellulosic hydrolysate as a low-cost carbon source on xylanase production by *T. pullulans*. Furthermore, to evaluate the effect of inhibitors generated during autohydrolysis pretreatment on fermentability of hydrolysates, two different hydrolysates detoxification methods were evaluated: XAD-7 and WA-30.

Different media preparations were formulated using the hemicellulosic liquid fraction obtained after the autohydrolysis and detoxification. *T. pullulans* was cultured in detoxified hemicellulosic hydrolysate (XAD-7 and WA-30), fermentation media (HH), supplemented with CSL, or mineral media supplemented with YE. Assays were done in 250 mL Erlenmeyer flasks at 20°C, inoculated with highly active cell culture, initial pH 5 and 150 rpm for 72 h. Samples were collected periodically for analysis. All experiments were run in duplicate.

Cell free extract was obtained by centrifugation at 13000xg for 10 min. The supernatant obtained was used as a crude extracellular xylanase enzyme for analytical purposes. Xylanase activity was determined by incubating supernatant with 1% (w/v) beechwood xylan in 0.05 M sodium acetate buffer pH 4.85 at 30°C. The reaction was interrupted with 3,5- dinitrosalicylic acid (DNS) reagent and released sugars were measured[10] using xylose as standard. Liquid fractions were also evaluated for sugar release by HPLC.

3 RESULTS & DISCUSSION

EUCALYPTUS RESIDUES COMPOSITION

The pinchips had the following chemical composition on dry basis: glucans 44.5 ± 1.7%, xylans 13.7 ± 0.8%, acid soluble and insoluble lignin 8.6 ± 0.5%, acetyl groups 4.4 ± 0.6%, extractives in water: EtOH 4.7 ± 0.5%, and ash 0.2 ± 0.1%.

DETOXIFIED AND NON-DETOXIFIED HEMICELLULOSIC HYDROLYSATE CHARACTERIZATION

As shown in Table 1, the adsorption resin XAD-7 was efficient in removing non-polar components such as lignin, whereas the anion-exchange resin WA-30 was able to remove more organic acids as well as some of the non-polar components due to its non-polar matrix.

Table 1. Composition of detoxified and non-detoxified autohydrolysis pretreated hydrolysate.

	Xylose (g/L)	Xylo-oligomers (g/L)	ASL (g/L)	AIL (g/L)	Acetic acid (g/L)	Formic acid (g/L)	Levulinic acid (g/L)
Hemicellulosic hydrolysate	2.2±0.1	12.6±0.6	2.0±0.1	0.48±0.02	1.3±0.1	2.5±0.1	0.20±0.01
Hydrolysate detoxified with XAD-7	1.9±0.1	6.7±0.4	0.3±0.1	0.01±0.00	0.7±0.1	1.91±0.1	0.10±0.10
Hydrolysate detoxified with WA-30	2.0±0.1	10.3±0.6	0.6±0.1	0.30±0.10	0.2±0.1	1.3±0.1	0.20±0.10

ASL: Acid soluble lignin; AIL: Acid Insoluble lignin (according to NREL protocols).

XYLANASE PRODUCTION

The effect of detoxification, YE and CSL on *T. pullulans* grown on hemicellulosic hydrolysate were studied by examining the biomass growth and the xylanase production. As shown in Figure 1, *T. pullulans* was able to grow and produce xylanases using the hemicellulosic hydrolysate as the sole carbon source. The results showed that there was no significant difference in xylanase production when either the eucalyptus hydrolysate media supplemented with CSL or the detoxified hydrolysates were used. Despite the fact that the detoxified hydrolysates evaluated, XAD-7 and WA-30, did not promote xylanase production over non-detoxified hydrolysates, WA-30 contributed to improve biomass growth (data not shown).

Although nitrogen sources such as CSL and YE have been reported to support microbial processes[11], CSL did not favour cellular growth (data not shown) or xylanase production over YE. The highest xylanase activity was 7.6 ± 0.4 IU/mL, detected at 48 h when *T. pullulans* was cultured in hemicellulosic hydrolysate supplemented with YE. This activity is comparable to xylanase activities reported for xylanase production in *P. sclerotiorum* (7.5 ± 0.1 IU/mL)[12] using lignocellulosic wastes.

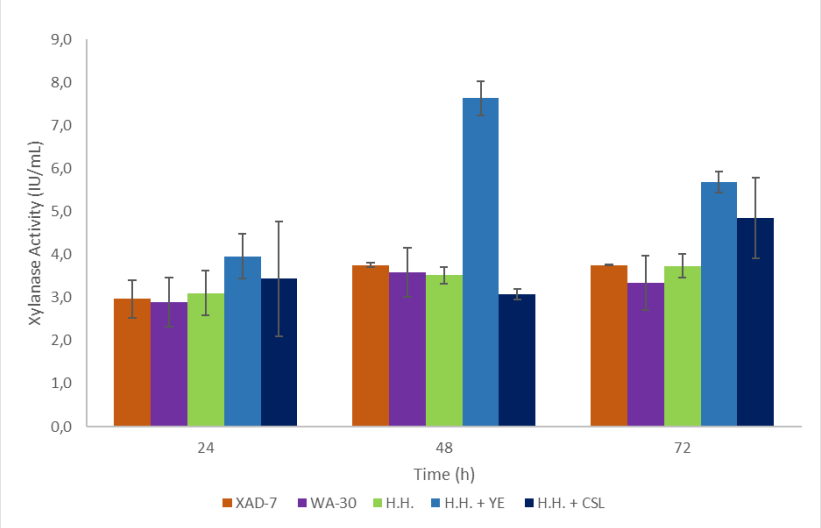

Figure 1. Extracellular xylanase activity of *T. pullulans* cultured in detoxified hydrolysate (XAD-7), detoxified hydrolysate (WAD-30), hemicellulosic hydrolysate (HH), supplemented with CSL (HH+CSL), mineral media supplemented with YE (HH + YE).

4 CONCLUSION

According to these results, xylanase production from hemicellulosic hydrolysate from hydrothermal pretreated industrial lignocellulosic residues is possible and the values are promising. The xylanase producer yeast used, *T. pullulans*, is an interesting candidate for processes that require low temperatures. Among the nitrogen sources evaluated, YE was preferred over CSL source for xylanase production. The culture medium with the hydrolysate without detoxification was selected for the xylanase production, which is advantageous because it lowers the cost of the medium production.

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ACKNOWLEDGEMENTS

Financial support was provided by the Agencia Nacional de Investigación e Innovación of Uruguay (FMV_1_2021_1_16777, POS_FMV_2021_1_1010847) and Biotechnology Postgraduate Programme of Facultad de Ciencias, UdelaR. The authors thank UPM Fray Bentos for supplying the wood pinchips used.