USE OF WHEAT BRAN FOR THE PRODUCTION OF β-GLUCOSIDASE, XYLANASE, β-XYLOSIDASE AND CMCase BY *Aspergillus awamori*

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ABSTRACT

In this study the insoluble substrate wheat bran was used, as carbon source to produce the enzymes β-glucosidase, xylanase, β-xylosidase and CMCase, that are of paramount importance for the degradation of lignocellulosic biomass into fermentable sugars. For that the fungus *Aspergillus awamori* was firstly cultivated in a pre-inoculum medium for two days and a portion of the liquid culture was transferred to the enzyme production medium. Both the pre-inoculum medium and the enzyme production medium presented wheat bran as carbon source. Nevertheless, industrial substrates such as wheat bran are economically attractive, scaling up enzyme production in submerged fermentation using a complex solid substrate is a challenge as the use of excessive wheat bran can cause problems in the operation of bioreactors. The results hereby obtained will be used in the next step of the work using a 2 L instrumented bioreactor.

Keywords: *Aspergillus awamori*, β-glucosidase, xylanase, β-xylosidase and CMCase enzymes production, submerged fermentation, wheat bran.

1 INTRODUCTION

Aspergillus awamori is an important fungus used for industrial enzyme production. It can be used in the production of biomass degrading enzymes due to its large enzyme secretion capacity. For industrial enzyme production, submerged fermentation is frequently used in complex media containing insoluble substrates. This fungus was used to produce biomass degrading enzymes and mostly β-glucosidase and xylanase, in growth medium presenting wheat bran as carbon source[1,2]. Wheat bran is a carbon source composed predominantly of non-starch carbohydrates which may induce xylanase production[2], besides starch and crude protein. It also presented yeast extract that may favor the accumulation of β-glucosidase.

Considering the subject of developing a bioprocess to produce second generation ethanol from sugarcane bagasse, the process patented by Silva *et al.* (2009) was considered as a reference. In this process, the enzymes produced by *A. awamori* are used in association with the enzymes produced by *T. reesei* to increase the production of fermentable sugars during the enzymatic hydrolysis of lignocellulosic biomass. Initial attempts to produce *A. awamori* enzymes in larger bioreactors (2L, 30L and 150L) were unsuccessful due to problems related to the excess of wheat bran present in the pre-culture, both during loading of the reactor and its operation. In this context, the objective of this study was to understand the effect of wheat bran on the production of β-glucosidase, xylanase, β-xylosidase and CMCase.

2 MATERIAL & METHODS

Cultures and spores of *A. awamori* were maintained and produced on standard on potato dextrose agar (PDA) for seven days at 30 ºC. Spore suspensions were obtained by addition of NaCl 0.9% (w/v) in Petri plates and subsequently lightly scraping the cultures. The suspensions were centrifuged for 15 min/6000 RPM and the spores were preserved in glycerol 20% (v/v) at -18 ºC.

Table 1 shows the formulations of the Breccia modified media[2] used for pre-inoculum and enzyme production (all with pH adjusted to 6.5), using different amounts of wheat bran as a carbon source. All pre-culture media (pH 6.5) were inoculated with 1 % v/v spore suspension (5.25×10^8 spores/mL), and the flasks were incubated for 2 days at 30°C and 200 RPM in a rotary shaker. The enzyme production was performed in Erlenmeyer flask of 500 mL, containing 150 mL of medium (table 1) and pH (6.5) was controlled using sodium phosphate buffer 0.1 M. After sterilization, duplicate cultures were inoculated with a 10% (v/v) of the pre-culture and then incubated for 7 days at 30 °C and 200 RPM in a rotary shaker. After 7 days, the contents of the flasks were centrifuged for 15 minutes at 6000 RPM, and the supernatant was used for enzymatic activity determination (β-glucosidase, xylanase, β-xylosidase and CMCase) using the methodology of the standardized operational procedures developed by the staff from Bioethanol Laboratory (https://bioetanol-ufrj.com.br/) based on NREL (National Renewable Energy Laboratory, USA) laboratory analytical procedures.

Table 1 Composition of the growth and enzyme production medium.

Components	Pre-culture medium (1)	Pre-culture medium (2)[2]	Enzyme production medium[2]
$NaNO_3$ (g/L)	3.0	3.0	3.0
Yeast extract (g/L)	10	10	10
Wheat bran (g/L)	**5.0**	**30**	**30**
C/N ratio	4.56	7.85	7.85

3 RESULTS & DISCUSSION

The activity of the enzymes produced using 5g/L and 30g/L of wheat bran in the pre-inoculum medium is presented in Figure 1.

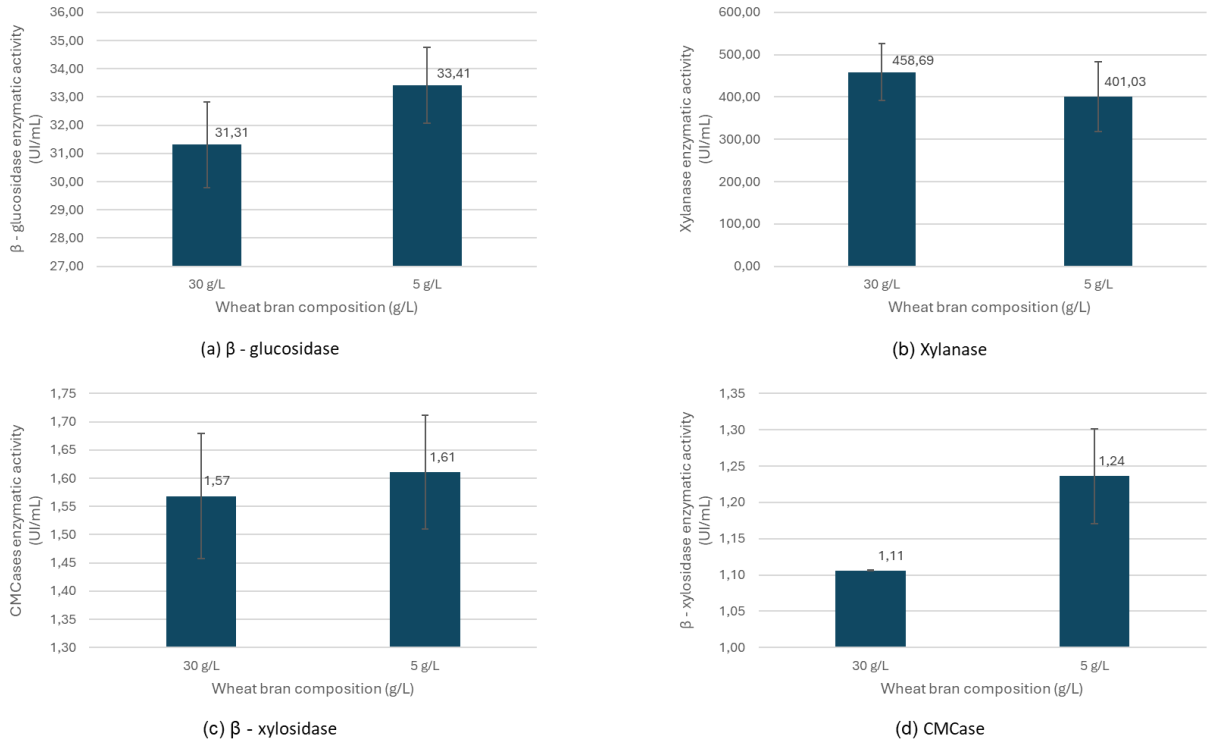

Figure 1 Enzyme activities in the culture supernatants upon 7 days of incubation: (a) β-glucosidase, (b) xylanase, (c) β-xylosidase and (d) CMCase. Pre-culture modified Breccia growth medium: 3.0 g/L $NaNO_3$, 10 g/L yeast extract and wheat bran (30 g/L or 5 g/L). Enzyme production medium: 3.0 g/L $NaNO_3$, 10 g/L yeast extract and 30 g/L wheat bran.

Excess of substrate hinders homogenization in the shake-flask fermentations, compromising fungal growth and enzyme production. Furthermore, the pre-inoculum incubation time (2 days) was not sufficient for the transformation of this amount of carbon source. Therefore, the reduction of wheat bran in the pre-inoculum cultivation medium was studied to assess its effect on enzyme production.

According to the results presented in Figure 1, *A. awamori* was able to produce the enzymes β-glucosidase, xylanase, β-xylosidase and CMCase in both pre-inoculum medium formulations (5 g/L and 30 g/L of wheat bran). However, enzyme concentrations were higher in the pre-inoculum medium presenting 5 g/L of wheat bran for the enzymes β-glucosidase (33.41 UI/mL), β-xylosidase (1.61 UI/mL) and CMCase (1.24 UI/mL). Equivalent accumulation of xylanase was observed for both medium, as 458,69 UI/mL and 401,03 UI/mL were measured for medium presenting 30g/L and 5g/L respectively. The enzyme levels that were observed were higher than in previous works conducted by solid-state fermentation[7]

Wheat bran is a carbon source composed predominantly of non-starch carbohydrates, starch, and crude protein. The non-starch carbohydrates are primarily arabinoxylans, cellulose, and β-(1-3) (1-4)-glucan[2], which may induce xylanase production (Fig1. (b)). In relation of β-xylosidase and CMCase, these enzymes reached low levels on submerged cultivation (Fig. 1 (c) e (d)), since they contribute to increase to the end products in the subsequent stage, the hydrolysis of sugarcane bagasse. The production of β-glucosidase was approximately 30% higher than similar studies available in the literature[6]. The next steps of this study involve the evaluation of wheat bran concentration in the enzymes production medium and its effect on the enzyme production.

CONCLUSION

The use of wheat bran was effective to produce the enzymes β-glucosidase, xylanase, β-xylosidase and CMCase by *Aspergillus awamori* using a wheat bran concentration of 5 g/L in pre-inoculum medium. These conditions will be further studied in instrumented bioreactors regarding its operational conditions and enzymes production cost using a higher wheat bran concentration.

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