BIOCONVERSION OF BREWERY'S SPENT MALT BY *ASPERGILLUS BRASILIENSIS*

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ABSTRACT

Agricultural and agro-industrial production are two of the most important socioeconomic activities in Brazil. Recently, the agro-industrial sector has started supplying raw materials for bioprocesses and bioproducts, such as the spent malt from breweries. In this study, the goal was to assess the potential of using *Aspergillus brasiliensis* BLf1 to convert brewery's spent malt into a suitable material for animal feed. In order to investigate this, a 96-hour solid state cultivation was conducted at a temperature of 30 ºC. During this period, the kinetics were assessed by measuring the concentrations of sugars and enzymatic activity. Samples were collected every 12 hours for duplicate cultivations by adding a sodium acetate buffer solution (50 mM, pH 5.0) to the culture flasks. After extraction, solids were separated by centrifugation and sugars and enzyme activity were determined in the supernatant solution. The results show that the concentration of total sugars peaked at 12 h, with 55.2 g/L and at 48 h of cultivation, with 44.4 g/L. Reducing sugars showed a decreasing behavior throughout the cultivation. Xylanase activity is maximum in 48 h with a value of 21.1 U/g of dry substrate and cellulase activity is maximum in 72 h with a value of 0.562 U/g of dry substrate. The enzymes cellulase and xylanase, along with changing sugar levels, indicate that *Aspergillus brasiliensis* BLf1 can alter the lignocellulosic matrix during solid-state cultivation on brewer's spent malt biomass. The findings indicate potential for using converted brewer's spent malt as animal feed.

Keywords: Agro-industrial byproducts, Lignocellulosic biomass, Solid-state cultivation, Xylanase activity, Animal feed.

1 INTRODUCTION

Agricultural and agro-industrial production are two of the most important socioeconomic activities in Brazil, providing a wide range of crops, livestock and processed products. However, this sector generates a substantial amount of residues and by-products, which can be used as substrates for bioprocessing and bioproducts. Spent malt is a byproduct of the brewing industry, being abundant in lignocellulosic material. Lignocellulose is the most available carbon resource in the world, and it is formed by long chains of cellulose interconnected by hydrogen bonds with intertwined hemicellulose molecules and protected by an external layer of lignin.[1] In addition to its use in producing lignocellulosic ethanol and other products, spent malt can also be potentially utilized as animal feed.[2] Nevertheless the residue might contain substances that hinder nutrient absorption, rendering it unsuitable as a raw food source for monogastric animals, as it does not promote their proper development. Physical, chemical and biological processes can treat lignocellulosic materials. Fungi can be used in biological treatments in order to reduce the degree of cellulose polymerization or partially hydrolyze the hemicellulose.[3] The increase in the porosity of the materials, the reduction in the crystallinity of cellulose, partial depolymerization of cellulose, solubilization of hemicellulose and the modification of the lignin structure are some of the effects of bioconversion.[4] *Aspergillus* fungi are well-suited for solid state cultivation. They efficiently colonize the substrate, mainly the porous regions, and appear to be less dependent on water activity.[5] The strain *Aspergillus brasiliensis* BLf1 has proven its capability to grow on multiple lignocellulosic substrates and has emerged as a significant producer of xylanases.[6] This research aims to evaluate the potential of using *Aspergillus brasiliensis* BLf1 to convert brewer's spent malt into a material suitable for animal feed.

2 MATERIAL & METHODS

The lignocellulosic biomass used as substrate in the study is brewer's spent malt. It was provided by Cervejaria Traum (Nova Petrópolis, Rio Grande do Sul, Brazil). The sample was dehydrated at 65°C for 48 hours. The filamentous fungus used as a cultivation agent in the study was the *Aspergillus brasiliensis* BLf1, which was isolated in the Amazonian environment and is deposited in the culture collection of the BiotecLab Laboratory (ICTA, UFRGS, Porto Alegre – RS).

The solid state cultivations were carried out in 250 mL Erlenmeyer flasks containing 5 g of substrate and mineral salt medium composed of 6 g/L $NaNO_3$; 0.52 g/L KCl; 0.52 g/L $MgSO_4.7\ H_2O$; 1.52 g/L KH_2PO_4; 1 mL of pyridoxine solution (100 mg/mL), 2 mL of traces of (g/L): 22 $ZnSO_4.7H_2O$; 11 H_3BO_3; 5 $MnCl_2.4H_2O$; 5 $FeSO_4.7H_2O$; 1.6 $CoCl_2.6H_2O$; 1.6 $CuSO_4.5H_2O$; 1.1 $(Mn_4)_6Mo7O_{24}$; and 50 EDTA, pH 5.8, to reach 60.9% final average humidity in the substrate. The flasks were sterilized by autoclaving at 121° C for 20 min, and inoculated with 3 discs (1 cm in diameter) of 1.8 x 10^6 spores each. Subsequently, they were incubated for 96 hours at 30 ±1 °C. Microorganism's growth was detected through visualization of mycelial growth. Samples were collected every 12 h, for cultivations in duplicate, by adding 40 mL of 50 mM sodium acetate buffer solution, pH 5.0, to the culture flasks. The flasks were shacked for 15 min at 120 rpm at 30°C. The solids were separated by centrifugation at 3,000 g at 4°C for 15 min.[6,7] Sugars and enzyme activity were determined in the supernatant solution.

Total sugars were determined colorimetrically, using the DNS method (3,5 Dinitrosalicylic Acid)[8]. The standard curve was constructed with glucose, varying concentrations from 0.1 g/L to 1 g/L. The absorbance reading was taken on a UV-visible spectrophotometer at 540 nm[8]. In order to complete the analysis, non-reducing sugars were quantified. In this case, hydrolysis was carried out with 2N HCl and then the medium was alkalinized with 2N NaOH. The DNS test was performed with the supernatant[9]. The quantification of arabinose, glucose and xylose were also carried out by high performance liquid chromatography.[6,7]

The enzymes were analyzed according to the standard procedure recommended by the IUPAC Biotechnology Commission[6]. The activity of cellulases and xylanases were assayed following a similar procedure. The enzymatic extracts obtained as described above were incubated with solutions of either 1% xylan or 0.8% carboxymethylcellullose solution as substrates, in 50 mM sodium acetate buffer solution, pH 5.0. The mixtures were left to react, and the dinitrossalicylic method test was performed with the supernatant. One unit of enzyme activity was defined as the amount of enzyme that produces 1 µmol of xylose or 1 µmol of glucose per minute. Activities were expressed as U/g of dry substrate.

3 RESULTS & DISCUSSION

The test was carried out with 60.9% final average humidity, pH 5.8. The ideal humidity for the development of fungi is between 60% and 70%. In their natural environment, fungi are found in environments where the pH varies from 2.0 to 9.0[10]. In this way, both the humidity and the pH of the cultivation are in the appropriate range for the development of the fungus. The concentration of total sugars peaked at 12 h, with 55.2 ± 5.9 g/L and at 48 h of cultivation, with 44.4 ± 3.2 g/L and then decreased over time, as shown in Figure 1. These peaks are due to the increase in the concentration of non-reducing sugars.

The decreasing behavior over time of reducing sugars is evident throughout the cultivation, starting with 48.5 ± 1.4 g/L and ending with 2.4 g/L. This was expected, as the fungus uses sugars to develop. Monosaccharides and disaccharides with at least one free anomeric carbon from the aldehyde group are also reducing agents, such as maltose, cellobiose and xylobiose.

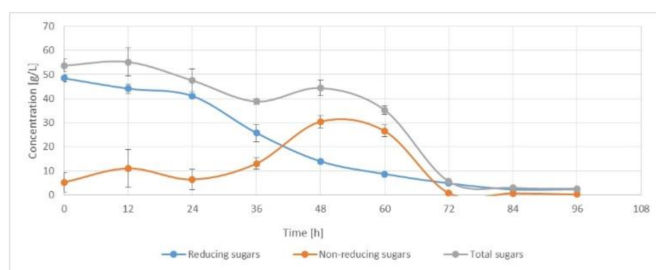

Figure 1. Total, reducing and non-reducing sugars during cultivation.

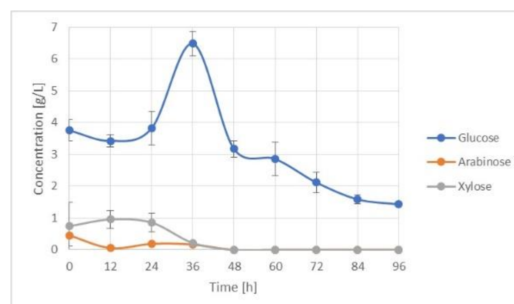

Figure 2 Arabinose, glucose and xylose during cultivation.

Non-reducing sugars peak at 48 h can be related to the presence of oligosaccharides, such as xylooligosaccharides (XOS), originating from the hydrolysis of xylan, a component of hemicellulose, through the action of xylanase enzymes. Menezes, Rossi and Ayub (2018) evaluated the presence of XOS in cultures using spent malt as a substrate and the fungus *Aspergillus brasiliensis* BLf1, and the highest concentration of XOS was also observed in 48 h of cultivation. This may occur because there is possibly still xylan present in the spent malt. However, after these peaks, there is a reduction in non-reducing sugars' concentration, which is explained by the natural process of action of the xylanolytic enzyme system. It consists of endoxylanases (1,4-β-D-xylan xylohydrolases), βD-xylosidases (1,4 β-xylosyl xylanhydrolases) and debranching enzymes (esterases). All of these enzymes act synergistically to convert xylan into its constituent sugars (reducing monosaccharides). The increase in xylose in the medium causes catabolic repression; therefore, there is a decrease in the production of endo- and exo-xylanases and thus a decrease in XOS through hydrolysis by β-xylosidases[11].

The identification and quantification of arabinose, glucose and xylose by HPLC is presented in Figure 2. Arabinose and xylose are present up to 48 h of cultivation, with maximum concentrations of 0.189 ± 0.013g/L and 0.957 ± 0.278 g/L. The xylanase activity (Figure 3), up to 12 h of cultivation, is not detected by the analysis. However, from 24 h onwards there is an increase in its activity, peaking at 48 h and with an activity of 21.1 ± 0.5 U/g of dry substrate. The difference in enzyme activity in relation to previous studies may be due to the difference in the characteristics of the substrate used and the variation in pH.[6]

Glucose is present throughout the culture, with a peak concentration at 36 hours with a value of 6.48 ± 0.38 g/L, possibly due to the degradation of cellulose by the fungus. Cellulase activity (Figure 4) is not identified until 36 hours of analysis. However, after 48 h of incubation there is an increase in its activity, peaking at 72 h and with an activity of 0.5619 ± 0.0364 U/g of dry substrate. The cellulase activity value is lower than the xylanase activity value. According to the literature, spent malt that has high concentrations of hemicellulose has a more amorphous structure and is more available in the lignocellulosic structure for the fungus to access, also due to the thermal treatments present in the industrial beer manufacturing process.

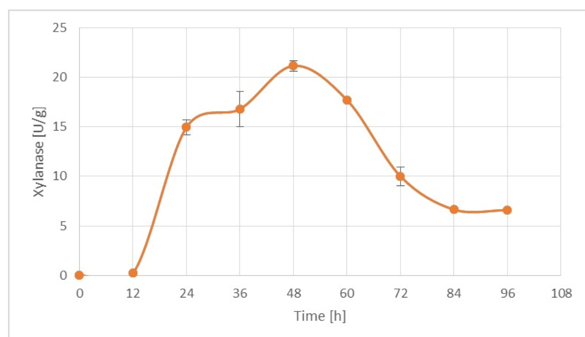

Figure 3 Xylanase activity during cultivation.

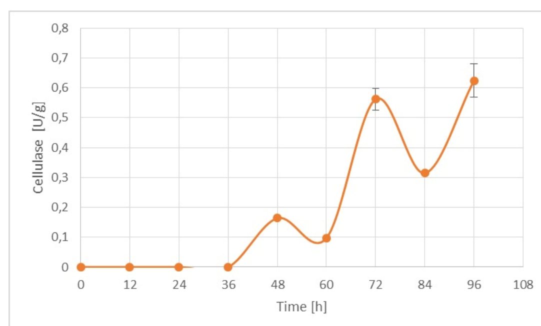

Figure 4 Cellulase activity during cultivation.

4 CONCLUSION

The activity of cellulase and xylanase enzymes, coupled with the variation in sugar concentration during the solid-state cultivation of *Aspergillus brasiliensis* BLf1 on brewer's spent malt, suggests that the fungus has the capacity to promote changes in the lignocellulosic matrix. The findings suggest that the converted brewer's spent malt shows promise as a material for animal feed, and further investigation is necessary to fully comprehend its potential.

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