**Screening of biomass degrading enzymes from *Aureobasidium pullulans*, using non-synthetic nitrogen sources**

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**Highlights**

* *A.pullulans* can be a cellulolytic enzyme producer
* Enzymes can be produced by non-synthetic medium
* Submerged fermentation showed to be a good method to produce these enzymes
* Soybean represent the best non-synthetic nitrogen source in this process

**1. Introduction**

With the increasing demand for renewable fuels and bioproducts, with a view to sustainable development, lignocellulosic biomass has become an important compound to be applied in bioprocesses due to its low cost and high availability. Brazil generates foreseeable amount of lignocellulosic biomass primarily agro-residues such as sugarcane bagasse (SB), sugarcane straw (SS), corn stover (CS) and *Eucalyptus* residues round the year (LYND *et al.* 2002). These lignocellulosic residues are excellent feedstock for second-generation (2G) sugars production which could be utilized for liquid fuels and value-added products such as biopigments, biopolymers and several other biochemicals based on biorefinery concept. However, for the cost competitive production of these products, the most important concern is the economic production of 2G sugars, as these sugars act as building block in biorefineries (FERNANDES, 2006). For 2G sugars production, efficient cellulases production and their concerted action on lignocellulosic residues is vital. Commecial cellulases are available in the market but due to their high costs, limited supply and monopoly of certain industries, this is necessary to focus on in-house production of stable and complete cellulolytic enzyme cocktails. The objective of this work was to study the production of cellulolytic enzymes from the microorganism *Aureobasidium pullulans* using sugarcane bagasse residues and soybean meal, corn and rice as a source of nitrogen.

**2. Methods**

 The investigations of the production of cellulase by the yeast *Aureobasidium pullulans* were carried out in order to evaluate sugarcane as carbon source using non-synthetic nitrogen source. Nonsynthetic nitrogen sources were soybean meal, corn bran and rice bran at 1.8 g/L, as well as for synthetic nitrogen sources in Mandel’s medium, which obtained a total weight of 1.8 g/L total. For the fermentation 1mL of the isolate stock was transferred to a pre-inoculum in 50mL of Sabouroud dextrose broth in shaker 30ºC and 200 rpm for 48 hours. Subsequently, 1.6x106 CFU were transferred to erlenmeyers containing 100 mL of the minimal Mandel medium with only one nitrogen source with 1% of the carbon source (sugarcane bagasse) and kept at 30°C for 96 h with shaking at 200 rpm. Samples were withdrawn every 24 hours with 5 minutes rest for decantation and 1mL of the supernatant was withdrawn for the reducing sugar test according to Ghose (1987).

**3. Results and discussion**

 The maximum activity also occurred during 60 h, for CMCase soybean meal was 6.865 U/mL and for FPase 2,449 U/mL corn meal and rice meal with CMCase of 3.692 U/mL and FPase of 1.407 U/mL, and CMCase of 3.842 U/mL and FPase of 1.425 U/mL, respectively. The figure shows the enzymatic activity as a function of the bioprocessing time from the use of organic nitrogen sources as the only factor of nitrogen in the process of submerged fermentation for the production of enzymes of the cellulolytic complex. These nitrogen sources have different proportion of proteins and sugars (MARTINIANO *et al*., 2017) and the results indicate that the protein source is important for the production of crude enzyme extract, however, not being the only factor, considering that these brans have very close amounts of protein (g/L). Ilmén *et al* (1997) questioned the action of nitrogen as an inducer in suggesting that the induction of nitrogen does not alter at the genetic level the production of enzymes, altering only the cell mass and, consequently, the production of enzymes. Non-synthetic nitrogen sources have been used in the literature to improve the cellulase synthesis conditions (AHAMED and VERMETTE, 2008), due to their low cost and to be, as well as sugarcane bagasse, a sub-agroindustrial product.

**4. Conclusions**

 The submerged fermentation of non-synthetic sources (soybean, corn bran and rice bran) can be used to improve the productivity of a CMCase and Fpase by using *A. pullulans* as producer. This nitrogen sources have advantages as low cost, availability and efficiency on submerged fermentation for cellulase production. This study is a preliminary study for further proteomic approach.

**References**

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