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Short communication

Biological pretreatment of sugar cane bagasse for the production of cellulases and xylanases by *Penicillium echinulatum*

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ABSTRACT

In this study, sugar cane bagasse was pretreated with the white rot fungus *Pleurotus sajor-caju* PS 2001, and this biomass was subsequently used in the production of cellulases and xylanases by the fungus *Penicillium echinulatum*. Despite the environmental advantages offered by this type of pretreatment, the enzymatic activity obtained with biologically pretreated sugar cane bagasse (PSCB) was lower than that of the control treatments, which were carried out with untreated sugar cane bagasse (SCB) and cellulose. For medium supplemented with PSCB, the average peak activities obtained were 0.13, 1.0, 0.18, and 0.33 U ml^{-1} for FPA, endoglucanase, β-glucosidases, and xylanases, respectively. For the cellulose, control values of 0.52, 1.20, 0.20, and 1.46 U ml^{-1}, and SCB values of 0.95, 1.60, 0.21, and 1.49 U ml^{-1} were obtained, respectively. Although the enzymatic activities of the culture with biologically pretreated sugar cane bagasse were lower than the cultures carried out with untreated sugar cane bagasse, it should be noted that production of enzymes of the cellulase and hemicellulase complex after production of the mushrooms is another way to add value to this agricultural residue.

1. Introduction

In view of current environmental problems in recent years, there has been an interest in the use of ethanol as vehicle fuel. Lignocelluloses are renewable materials that are attractive for the production of ethanol since they are abundant and inexpensive (Wheals et al., 1999). One lignocellulose found in great quantities, especially in tropical countries, is sugar cane bagasse, a byproduct of the production of sucrose and ethanol (Martín et al., 2007).

However, the polysaccharides (cellulose and hemicellulose) present in the lignocellulose biomass need to be hydrolyzed with acids or enzymes in order to liberate fermentable sugars. In many processes in the enzymatic conversion of lignocellulose biomass to ethanol and other chemical products, a pretreatment stage is required to break the lignin structure and to partially solubilize the polysaccharides (Keller et al., 2003).

Several pretreatment methods have been extensively investigated, such as steam explosion, solvent extraction, and thermal pretreatment using acids or bases (Mosier et al., 2005), along with biological pretreatments with white rot fungi (Itoh et al., 2003).

Many pretreatment processes require expensive equipment and large quantities of energy. In the physical processes, energy expenditure can make the conversion of holocellulose

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economically unviable (Wingren et al., 2004). Also, in pretreatments carried out by steam explosion, the glucose liberated from the cellulose can be degraded in the 5-hydroxymethylfurfural and levulinic and formic acids, while the pentoses liberated from the hemicellulose can be converted to furfural and formic acid. These products act as inhibitors in the later stages of enzymatic hydrolysis and fermentation (Klinke et al., 2003).

Chemical pretreatments have several disadvantages, notably the need for equipment made with corrosion-resistant materials, extensive biomass washings, and consequently, the generation of residues with reagents. Biological pretreatments using an efficient and environmentally correct method for the removal of lignocellulose lignin have been previously reported (Itoh et al., 2003). The most promising microorganisms for biological pretreatment are the white rot fungi, belonging to the class Basidiomycetes (Pan et al., 2005; Okano et al., 2005).

In this study, sugar cane bagasse (SCB) was biologically pretreated with the white rot fungus *Pleurotus sajor-caju* PS2001. Subsequently, the use of this biologically pretreated bagasse (PSCB) was evaluated for the production of cellulases and xylanases by the fungus *Penicillium echinulatum*.

2. Materials and methods

2.1. Microorganisms and culture media

The cellulose mutant *P. echinulatum* strain 9A02S1 (DMS 18942) was used for the production of enzymes. This strain was obtained by exposing the wild strain *P. echinulatum* 2HH to ultraviolet light and hydrogen peroxide (Dillon et al., 2006). For biological pretreatment, the fungus *P. sajor-caju* PS 2001 was used.

The strain *P. echinulatum* 9A02S1 was grown and maintained in cellulose agar (C-agar). This medium contained 1% (v/v) of soaked cellulose, 10% (v/v) salt medium (Mandels and Reese, 1957), 0.1% (w/v) proteose peptone (Oxoid L85), and 2% (w/v) agar. The cultures were grown for 7 days at 28 °C until the formation of conidia, after which they were stored at 4 °C until use. The medium used for the maintenance and preparation of the inoculums of the strain *P. sajor-caju* PS2001 contained 2% (w/v) of ground and soaked *Pinus* sp. sawdust, 2% (w/v) ground wheat flour, 0.2% (w/v) calcium carbonate, and 2% (w/v) agar.

2.2. Biological pretreatment

Biological pretreatment was carried out with the white rot fungus *P. sajor-caju* PS2001. A medium was prepared for the culture comprised of 94% (w/w) of sugar cane bagasse, with 5% wheat bran and 1% calcium carbonate added. This medium was supplemented with 100 ppm $(NH_4)_2SO_4$, 0.1 ppm $MnSO_4{\cdot}H_2O$, and 0.1 ppm $CuSO_4{\cdot}H_2O$. The humidity was adjusted to 66%.

Polypropylene bags with dimensions 17.8 cm × 13.0 cm containing 500 g of wet medium were used as microfermentors. These were sterilized by autoclaving at 1 atm for 2 h and were inoculated with a mycelial discs 1 cm in radius, cut from cultures grown for 7 days on plates with the inoculation medium. The cultures were maintained in an environment suitable for mycelial development, at 25 ± 1.5 °C and in the dark for 45 days.

After the development of mycelia over the entire substrate to allow the formation of fruiting bodies, small holes were made in the microfermentors and these were placed in an environment suitable for fruiting, at 20 ± 2 °C, with a photoperiod of 12 h at 90% humidity. After the fruiting, the residues of the microfermentors were collected, sieved, and used in the production of cellulases.

2.3. Production of enzymes

The submersed cultures were carried out in 500 ml Erlenmeyer flasks containing 100 ml of the production medium comprised of 10% (v/v) of salt medium (Mandels and Reese, 1957), 1% (w/v) of sugar cane bagasse pretreated with *P. sajor-caju* PS2001 (PSCB), 0.2% soy bran, 0.1% (w/v) Tween 80, and 90% of distillated water. In the control cultures, cellulose (Cel) and untreated bagasse (SCB) were used.

The flasks were inoculated with 1×10^5 conidia ml^{-1} and kept under shaking at 180 rpm, at 28 °C, for 6 days. The samples were removed at different time intervals and filtered. The enzymatic broth was collected for determination of enzymatic activity, and the experiments were carried out in triplicate.

2.4. Enzyme dosage

Determination of the filter paper activity (FPA) was carried out according to Ghose (1987). The β-glucosidase activity was dosed using salicine as the substrate, according to Chahal (1985). The endoglucanase was determined according to Ghose (1987) using a 2% carboxymethylcellulose solution in citrate buffer at pH 4.8. The xylanase activity was determined according to Bailey et al. (1992). The reducing sugars were dosed by the method of Miller (1959) with 3,5-dinitrosalicylic acid (DNS). One unit of enzymatic activity was defined as the quantity of enzyme required to liberate 1 μmol of reducing sugars of the substrate, given per ml of enzymatic filtrate under the analysis conditions.

2.5. Analytical methods

The quantity of N-acetylglucosamine was determined by the method described by Reissig et al. (1955), the quantity of mycelial mass was determined according to Bittencourt et al. (2002), and the reducing sugars were hydrolyzed with 10 M sulfuric acid, neutralized with sodium hydroxide, and dosed with DNS using the method of Miller (1959).

2.6. Statistical analysis

The results were analyzed statistically using analysis of variance with the Tukey's post-test for $p < 0.05$, using the PrismGraphPad software program.