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Detoxification procedures of eucalyptus hemicellulose hydrolysate for xylitol production by *Candida guilliermondii*

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Abstract

Xylitol can be obtained from hemicelullosic fraction of lignocellulosic materials containing D-xylose. A problem associated with the fermentation of hemicellulosic sugars to xylitol is the presence of a broad range of compounds, which inhibit the fermenting microorganisms. These inhibitors can be removed from the hydrolysate by a detoxification method, prior to fermentation. This study describes different detoxification methods of eucalyptus residues hydrolysate to improve xylitol production by *Candida guilliermondii*. The changes in the concentrations of fermentable sugars and four groups of inhibitory compounds were determined and the fermentability of detoxified hydrolysates was assayed. The applied detoxification methods include: treatment with active charcoal and four different resins (cationic and anionic) in sequence. Ion exchange resins were more efficient than activated charcoal to remove all four major groups of inhibitory compounds without sugar loss. The ion exchange detoxification drastically enhanced the fermentability of the hydrolysate. Under the best assayed operating conditions, 32.7 g/L of xylitol were achieved after 48 h fermentation, which correspond to 0.68 g/L h volumetric productivity and 0.57 g/g xylitol yield factor. In addition, it was verified that low concentrations of acetic acid can have a benefical effect on xylitol productivity.

Keywords: Xylitol; Xylose; Detoxification; Eucalyptus residues hydrolysate; Ion exchange resins; Activated charcoal

1. Introduction

Xylitol, a natural five-carbon sugar alcohol with sweetness comparable with that of sucrose [1] has found increasing use in the food and medical–pharmaceutical industries, due to several advantages. It has anticariogenic properties, is appropriate to replace glucose in diets of diabetics and has been recommended for parenteral nutrition and to prevent otitis, osteoporosis and lung infections [1,2].

The hydrolysate of hemicelullosic fraction of lignocellulosic materials, containing D-xylose, can serve as substrate for xylitol production by chemical or biotechnological means [3]. Nowadays, xylitol is obtained by chemical reduction of D-xylose, but expensive separation and purification steps are necessary to remove inhibitors from xylose or xylitol syrups. On the other hand, xylitol can be also microbiologically produced from xylose solution obtained by hydrolysis of lignocellulosic materials [3,4].

Brazil has a highly developed forest plantation state. These plantations are dominated by eucalyptus species with an estimated planted area ranging from 2.96 to 3.6 million hectares [5]. These numbers will even increase with future advances in genetic, cloning, fertilization and management. However, approximately one third of the total dry mass of eucalyptus is left in the field in form of branch, leaves, bark, etc. [5]. The acid hydrolysis of eucalyptus wood results in hydrolysate with large amounts of fermentable sugars. In this way, different researchers have employed eucalyptus hydrolysates for xylitol production [4,6,7]. Nevertheless, a common problem associated with efficient conversion of hemicellulosic sugars to xylitol by microorganism is that the hemicellulosic hydrolysate contains a broad range of compounds, which inhibit the fermenting microorganisms like *Candida guilliermondii* [4,7]. These inhibitors can be present in the raw material or be produced during the hydrolysis process. They are classified in four major groups: sugar-derived by-products (furfural and hydroxymethylfurfural), aliphatic acids (acetic acid, levulinic acid), lignin degradation products which include a wide range of aromatic and polyaromatic compounds with a variety of substituents, and inhibitors derived from metals or minerals in wood, soil

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or hydrolysis equipment [7]. The nature and concentrations of final inhibitory compounds vary greatly with the hydrolysis conditions and the used raw materials [4].

In the production of xylitol from lignocelullose, the problem with fermentations inhibition can be overcome by the hydrolysate detoxification prior to fermentation. Several methods have been employed, including yeast adaptation [8,9], neutralization and overliming [6], evaporation [10,11], solvent extraction [12,13], charcoal adsorption [14–16], biological treatment [17,18] and the use of ion exchange resin [19,20]. Among these methods, the charcoal adsorption and the ion exchange resin have been reported as the most efficient [7,19].

Adsorption with activated charcoal is a well-established detoxification method for sugar cane bagasse and wood hydrolysates [16,21]. According to the results, the production of wastes and the inviability of its reuse had been appointed as the principal drawback of charcoal method [10]. On the other hand, studies on detoxification efficacy show that ion exchange treatment in batch procedures can significantly improve the fermentability of lignocellulosic hydrolysates [19,22]. Ion exchange processes have been extensively used to reduce the concentration of toxic elements in many other areas of technology and are usually practiced in columns. Ion exchange columns are easy to use and more efficient than batch procedures. It differs from other detoxification methods due to its capacity to remove organic and inorganic compounds [19]. Besides, ion exchange resins have the advantage that they can be regenerated and reused without affecting the efficiency of the treatment, thus decreasing the overall cost of the process.

The more common hemicellulosic eucalyptus hydrolysates are prepared from wood-chips [4,13,14], a waste product of normal forestry operations. Differently, eucalyptus residues collected directly from landfill were used as raw material for hydrolysis in this work. The principal characteristic of this material is a high heterogeneity composition. Therefore, the present study deals with the evaluation of eight methods of detoxification of hydrolysate from such eucalyptus residues, for xylitol production. The analyses were performed to characterize the chemical effect of the detoxification methods on the four major groups of inhibitory compounds. The efficiency of each method was subsequently assayed in fermentations with *C. guilliermondii.*

2. Materials and methods

2.1. Raw material

Eucalyptus grandis residues were kindly supplied by Cia. Suzano de Papel e Celulose, São Luis de Paraitinga, SP, Brazil. The residues were comminuted, homogenized, air dried and stored. The average composition, determined according to methodology previously described by Ferraz et al. [23], was 40.2% cellulose, 15.7% hemicellulose, 26.9% lignin, 1.23% extractives and 1.4% ash.

2.2. Preparation of hemicellulosic hydrolysate

Acid hydrolysis was conducted in a 50 L reactor as previously described by Canettieri et al. [24] with H_2SO_4 (0.65%), at 157 °C, for 20 min, using 1:8.6 liquid/solid ratio. The solid and liquid phases were separated by filtration. The liquid phase, thereafter named as hydrolysate, was concentrated four to five-fold by evaporation at 70 °C, under vacuum. The raw and concentrated hydrolysates were chemically characterized.

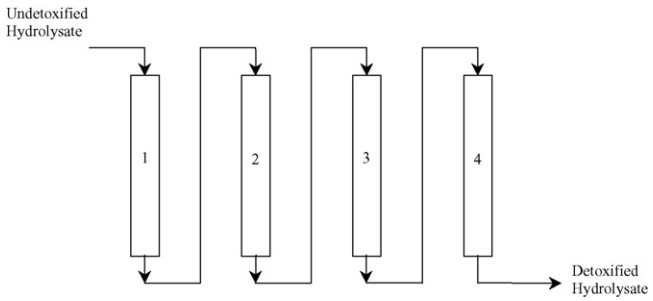

Fig. 1. Schematic representation of the hydrolysate detoxification with ion exchange resins: (1) cation exchanger type Applexion in H^+ form (Applexion Inc., USA); (2) anion exchanger type A-860S in Cl^- form (Purolite International, USA); (3) cation exchanger type C-150 in H^+ form (Purolite International, USA); (4) anion exchanger type Applexion in OH^- form (Applexion Inc., USA).

2.3. Detoxification procedures

2.3.1. Activated charcoal adsorption

The concentrated hydrolysate was treated with commercial CaO until pH 7, filtered and acidified to pH 5.5 or 1.8 with sulfuric acid. The solutions were kept at cool chamber (4 °C), for 24 h and then the precipitate was removed by centrifugation. After centrifugation, the solutions were mixed with powered charcoal (CDA, BRASILAC, Brasil) at 1% for 30 min or 5% (w/v) for 30 or 60 min and stirred (100 rpm) at 30 °C. The liquor was recovered by filtration, chemically characterized and used for culture media.

2.3.2. Ion exchange resins

The concentrated hydrolysate was subjected to column ion exchange using a four-resin system at room temperature as shown in Fig. 1. The effect caused by some operational characteristics like flow rate and flow mode (down flow or reverse flow) and previous hydrolysate pH adjustment was assayed.

Prior to the treatment, the hydrolysate pH was adjusted to 1.8 or to 5.5 using anion exchanger type A-103S, in OH^- form (Purolite International, USA). A 400 mL bed-volume and an average flow rate of 10 or 6 mL/min, down flow or up flow were used for detoxifying the hydrolysate in all the ion exchange resins. After saturation, determined by breakthrough curves, all the resins were washed with three bed-volumes of distilled water at an average flow rate of 1.5 mL/min and then regenerated according to the manufacturer recommendations. The obtained final liquor was characterized and used for culture media.

2.4. Microorganism and growth conditions

2.4.1. Microorganism and inoculum preparation

C. guilliermondii FTI 20037, described by Barbosa et al. [25], was maintained on agar malt extract slants at 4 °C. A loopful of cells was transferred to 500 mL Erlenmeyer flasks containing 200 mL of medium consisting of (g/L): xylose (30.0), glucose (2.0), ammonium sulfate (3.0), calcium chloride (0.1) and rice bran extract (20.0). The cells were incubated for 24 h in an orbital shaker (200 rpm, 30 °C), collected by centrifugation (2000 × *g*, 15 min), washed and resuspended in sterile distilled water.

2.4.2. Medium and fermentation conditions

The raw and detoxified hydrolysates were sterilized in autoclave (111 °C for 15 min) and supplemented with ammonium sulphate (2.0 g/L) and rice bran extract (20.0 g/L). All fermentations were carried out in an orbital shaker at 200 rpm, 30 °C, in 125 mL Erlenmeyer flasks with 50 mL of medium. The flasks were inoculated to an initial cell mass concentration of 3.0 g/L. The pH of the media was set to 5.5 at the beginning of the fermentation. Fermentation of a solution containing xylose in the same concentration as in the hydrolysates and supplemented with the same nutrient was performed as reference. At given