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Strategies of detoxification and fermentation for biotechnological production of xylitol from sugarcane bagasse

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ABSTRACT

The aim of this study was to evaluate strategies of detoxification and fermentation of the hemicellulosic liquors obtained from sugarcane bagasse autohydrolysis, for the biotechnological production of xylitol. Different sequences of detoxification treatments were performed, and their effects on sugars loss and inhibitors removal were evaluated. Fermentation assays were accomplished with commercial xylose to select the best yeast (*C. guilliermondii*, *C. tropicalis*) and the fermentation conditions of the detoxified spent liquors. Detoxification through a sequence of treatments, including $Ca(OH)_2$, IR-120 resin, activated charcoal, and IRA-67 resin, practically removed all inhibitors from the hemicellulosic spent liquors. Maximal concentration of xylitol obtained was 32.0 g L^{-1} (*C. tropicalis*, xylose: 104.1 g L^{-1}, yield: 0.46 g g^{-1}, productivity: 0.27 g L^{-1} h^{-1}). The technological parameters to obtain a detoxified spent liquor rich in xylose and its bioconversion to xylitol were determined.

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1. Introduction

Developing countries are in search of strategies for converting their production technologies so as to open up new markets and improve regional economies. The final disposal of lignocellulosic wastes generated during agricultural raw materials processing (sugarcane bagasse, rice husks, stalks of sunflowers, etc.), creates environmental problems associated with burning and/or accumulation. Therefore, one of these strategies is based on the use of lignocellulosic waste from agroindustrial raw materials to obtain high value products through the application of various conversion processes.

Sugarcane bagasse is the lignocellulosic waste generated in sugar mills (180–280 kg bagasse per ton of sugarcane) and constitutes an important source of renewable material, available in large quantities at low cost (Rao et al., 2012). Its composition depends on the climatic and soil conditions in which sugarcane has grown, but a typical composition is: 43–45% cellulose, 21–23% lignin, 25–32% hemicelluloses (mainly xylans) and minor amounts of extractive and ash (Area et al., 2012; Vallejos et al., 2012).

Xylans are composed of xylose units and may contain different substituents in the chain. They can be depolymerized into xylose, a primary carbon source for the bioproduction of xylitol, ethanol, and others. Xylitol is used as a sweetening agent and it is industrially produced by chemical reduction of xylose by expensive processes (De Albuquerque et al., 2014; Hou-Rui, 2012; Rao et al., 2012). Xylitol can also be produced biotechnologically by xylose conversion using specific microorganisms, being a metabolic intermediate product of xylose (Ikeuchi et al., 1999; Sasaki et al., 2012). The yeasts which have shown the highest yields of fermentation of xylose to xylitol are: *Candida guilliermondii*, *Candida tropicalis*, *Candida boidinii*, *Candida parapsilosis* and *Pichias* (Rafiqul and Sakinah, 2013).

The biotechnological process represents a lower cost alternative to the chemical one, as it is performed at atmospheric pressure and the purification of hydrolysates is less complex (Canilha et al., 2006; Rafiqul and Sakinah, 2013). Studies on culture medium prepared from lignocellulosic hydrolysates are focused on the removal of compounds that cause inhibition of microbial metabolism and decrease cell growth and product yield (Rafiqul and Sakinah, 2013). Some degradation products of sugars and lignin can adversely affect the fermentation process because they are toxic to microorganisms and inhibit their metabolism. Factors affecting xylitol production are: initial inoculum concentration, type of substrate, composition of culture medium, inhibitor compounds, temperature, pH, and oxygen transfer (Rao et al., 2004).

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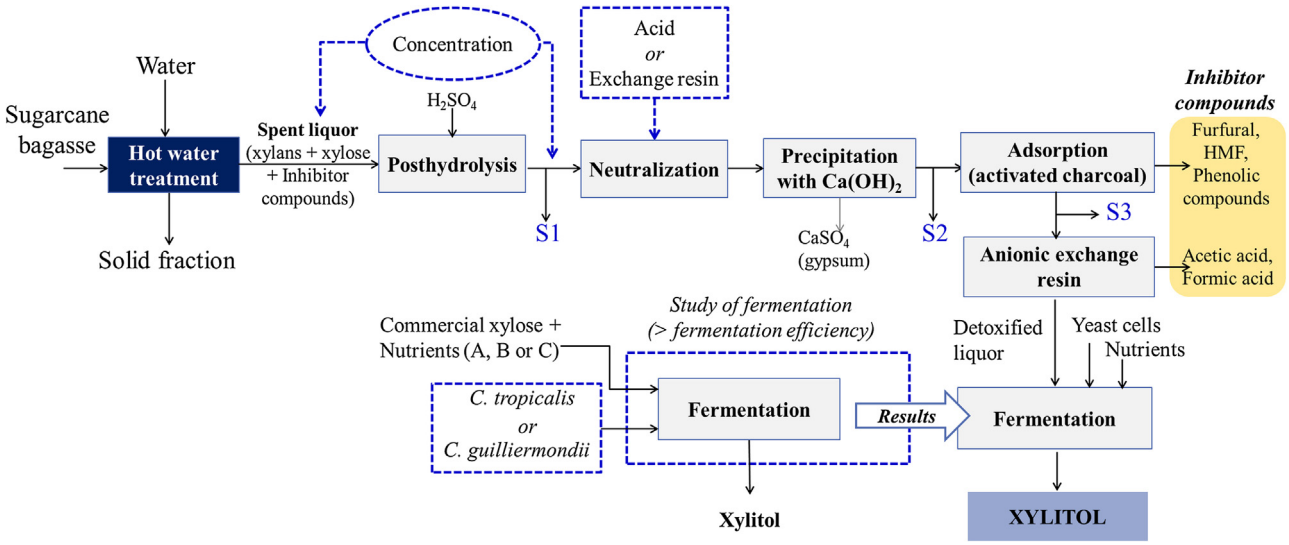

Fig. 1. Complete methodology used to obtain xylose and to convert it to xylitol (dotted line: alternatives).

Hot water treatment, named also autohydrolysis, is effective for hemicelluloses solubilization. This technology is environment friendly as it does not use any chemicals other than water (Garrote et al., 1999). In this process (aqueous medium, 150–250 °C, pH between 3 and 4), the hemicelluloses are hydrolyzed to oligo and monosaccharides (Caparrós et al., 2007; Vallejos et al., 2015). These conditions also promote the formation of low molecular weight aliphatic acids, furfural, and hydroxymethylfurfural (Jönsson and Martín, 2016). Lignin undergoes reactions of degradation and repolymerization, generating partially water soluble phenolic derivatives and insoluble condensation products, respectively (Rafiqul and Sakinah, 2013). Despite their low concentration, these compounds may act as inhibitors of microorganisms in the fermentation of hydrolysates from lignocellulosic materials (Larsson et al., 2000).

The concentration of inhibitors and sugars in spent liquors depends on raw materials and processing conditions. Detoxification may be accomplished by several methods, namely: (i) vacuum evaporation, (ii) ion exchange resins, (iii) activated charcoal, (iv) enzymes or microorganisms, (v) extraction with ether or ethyl acetate, and (vi) alkaline neutralization and precipitation. Detoxification methods cannot be compared with each other when different spent liquors and microorganisms are used, since liquors may have different amount and type of inhibitors and microorganisms present different tolerances to them (Jönsson et al., 2013). The identification and quantification of each compound is difficult because of the numerous and various aromatic compounds that can be found in different lignocellulosic hydrolysates.

Xylose sources and conversion treatments are keys to allow a cost-effective production of xylitol. Some strategies were explored to produce xylitol in an economical and environmentally friendly way (Clauser et al., 2015; Franceschin et al., 2011; Hernández et al., 2014). Because of the dissimilar prices of xylitol and ethanol, the co-production of xylitol with ethanol may improve the viability of lignocellulosic biorefineries, making feasible their installation in small-scale (Rao et al., 2012; Rueda et al., 2016). Nevertheless, there are few reports about an integral study of the processes involved in xylose extraction from sugarcane bagasse, its purification, and its conversion to xylitol (Rao et al., 2006; Silva et al., 2005).

The aim of this study was to evaluate strategies of detoxification and fermentation of the hemicellulosic spent liquors from sugarcane bagasse autohydrolysis for the biotechnological production of xylitol.

2. Materials and methods

2.1. Raw material

Sugarcane bagasse was collected in a local sugar mill (San Javier Sugar Mill, Misiones, Argentina). Bagasse pith was removed in two stages. In first stage, bagasse was wet-depithed to break its structure in a Bauer disc refiner (plate gap of 0.01 in), after which the bagasse pith was removed by screening, in a second stage, using a plate with 2 mm wide slits (Wenmber). Finally, depithed bagasse was centrifuged and preserved in a refrigerator. The bagasse was characterized in a previous work (Vallejos et al., 2015). The complete methodology used in this work for obtaining xylose and for converting it to xylitol is schematized in Fig. 1x.

2.2. Autohydrolysis of sugarcane bagasse and post-hydrolysis detoxification of spent liquors

The sequential study of autohydrolysis, post-hydrolysis and detoxification treatments is described below and shown in Fig. 2.

2.2.1. Autohydrolysis of sugarcane bagasse

Sugarcane bagasse was treated with hot water under isothermal conditions at 180 °C and 20 min. The hot water pretreatment conditions were selected on the base of results obtained in a previous study about the kinetic study of the extraction of hemicellulosic carbohydrates from sugarcane bagasse by this kind of treatment. A detailed description of the treatment can be found in Vallejos et al., 2015. The chemical composition of sugarcane bagasse was: 43.1% glucans, 23.8% xylans, 1.7% arabinans, 1.7% acetyl groups, 21.3% lignin, 4.8% extractives, and 1.5% ash.

Hot water pretreatments were performed with two different liquid-solid ratios (LSR) to obtain dilute and concentrated liquors. Dilute liquor was obtained using LSR of 14:1 in a MK digester (7 L) with a recirculation system using 350 g OD of bagasse, whilst concentrated liquor was obtained using LSR of 4:1 in a multipurpose reactor (4 L) heated by direct steam without stirring, using 300 g OD of bagasse. The extraction of xylans (wt.%) in the pretreatments and the chemical composition of the liquors were determined.

2.2.2. Post-hydrolysis of spent liquors

Hot water pretreatment produces partial solubilization of xylans mainly as *xylo*-oligomers, therefore post-hydrolysis of spent

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