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Sono-assisted alkaline pretreatment of sugarcane bagasse for cellulosic ethanol production

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ABSTRACT

The sono-assisted alkaline pretreatment of sugarcane bagasse was optimized using a central composite design in which the glucose recovery after enzymatic hydrolysis was used as the response function. Cellic® CTec3 (Novozymes) and Thermossac® Dry (Lallemand) were used for enzymatic hydrolysis and fermentation, respectively. The best performance was achieved at 70 °C with 140 W of sonication power using 0.125 g of NaOH g^{-1} of dry biomass, yielding a total glucose recovery of 95.8 wt% in relation to the total glucan content of the original material. The fermentation efficiency of the best substrate hydrolysate was 91.4%, whereas lower values of 87.1 and 80.0% were obtained for steam-exploded and sono-assisted alkali-washed steam-exploded cane bagasse, respectively. In addition, when the glucan recovery of each pretreatment method was taken into account, the total C6 cellulosic ethanol production from the sono-assisted alkali-washed cane bagasse was 20.4 and 40.6% higher than that of the other two pretreatment technologies, respectively.

1. Introduction

Brazil is the largest producer of sugarcane in the world [1,2] and its industrial processing generates several by-products such as bagasse, straw, molasses, filter cake and residual water [3]. The annual production of cane bagasse in Brazil is around 186 million tons [4]. Today, most of this lignocellulosic material is used as the main energy source in both sugar and ethanol mills including bioelectricity in advanced cogeneration facilities [5]. However, there is a surplus that is readily available as a sustainable resource for biorefineries, particularly for the production of ethanol, animal feed, furan compounds, and fibers for structural materials, composites, and pulp and paper [6,7].

The sugarcane bagasse, as any other type of lignocellulose, is primarily composed of cellulose, hemicelluloses and lignin [8,9]. Hence, due to the close association that exists among these plant cell wall components, a pretreatment method is required for its efficient use as a raw material for ethanol production, which includes the need for high accessibility to enzymatic hydrolysis and good fermentation yields. This is so because the enzymatic hydrolysis of cellulose and other plant cell wall polysaccharides is restricted by several factors including the substrate recalcitrance, which involves properties such as available surface area, pore volume, cellulose crystallinity, lignin content, and fiber coating by non-cellulosic components. These and other parameters define the enzymes' accessibility to the substrate and their ability to release fermentable sugars from plant polysaccharides [10].

Different pretreatment methods have been proposed so far to deconstruct the plant cell wall structure, remove and/or modify both lignin and hemicellulose components, and enhance the accessibility of glucans to enzymatic hydrolysis, this without compromising the fermentation yield by releasing inhibitory compounds in the reaction system [12–14]. In this regard, the pretreatment step has a strong influence in the overall process costs by determining the extent of sugar recovery, fermentation toxicity, enzymatic hydrolysis rates, enzyme loadings, and other process variables [11].

A number of pretreatment options are available: dilute acid, alkaline extraction, steam explosion, ammonia fiber extraction, wet oxidation, organosolv, and hot water extraction [15–17].

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Alkaline pretreatments involve delignification using dilute sodium hydroxide, potassium hydroxide, ammonia or lime, usually under conditions milder than those of other pretreatment processes [7]. This type of pretreatment consists of applying a basic solution to solubilize lignin–carbohydrate complexes by the breakdown of alkaline labile aryl–ether bonds (e.g., α—O—4 and β—O—4) and by acid-base reactions involving both aliphatic (mostly due to carbohydrates) and aromatic hydroxyl groups (exclusively due to the polyphenolic structure of lignin). In general, this extraction procedure decreases both lignin and hemicellulose contents, modifies the substrate structural organization (including cellulose crystallinity), and induces biomass swelling, thereby improving the substrate susceptibility to enzymatic hydrolysis [18]. In addition, the alkaline pretreatment produces less inhibitory compounds to enzymes and fermenting microorganisms compared to pretreatments based on acid hydrolysis [19].

Physical pretreatment methods such as ultrasonic radiation have also been applied to improve the efficiency of alkaline washing. The frequency at which the radiation is emitted produces a cavitation phenomenon that increases the temperature and pressure of aqueous reaction systems. For instance, the cavitation phenomenon produces free radicals from water molecules that are capable of cleaving bonds between lignin and hemicelluloses. Subhedar and Gogate [20] showed that the use of ultrasound reduces the alkali requirement and the processing time for delignification, whereas for Velmurugan and Muthukumar [21], the most attractive features of the sono-assisted alkaline pretreatment were the substantial reductions in pretreatment time and temperature, although the substrate accessibility to enzymatic hydrolysis was also improved considerably.

Besides the alkaline extraction, steam explosion is one of the most widely employed physicochemical pretreatment methods for the fractionation of lignocellulosic materials such as cane bagasse and this has been demonstrated in both laboratory and pilot scale [5]. Depending on the pretreatment severity, this method is able to remove the hemicellulose component almost completely and to impart chemical modifications to the lignin component so as to produce cellulosic materials that are highly accessible to enzymatic hydrolysis [22]. However, due to the acid environment in which steam explosion is performed, water soluble furan compounds and organic acids are released and these tend to inhibit the optimal performance of both enzymes and fermenting microorganisms [23].

In this work, the sono-assisted alkaline pretreatment of sugarcane bagasse was optimized using a central composite design in which the glucose recovery after enzymatic hydrolysis was used as the response function. Afterwards, enzymatic hydrolysates derived from the best alkali-treated substrate were fermented by an industrial strain of *Saccharomyces cerevisiae* and the resulting ethanol yields were compared with those derived from other pretreatment technologies such as steam explosion with and without post-delignification with dilute sodium hydroxide.

2. Material and methods

2.1. Material

Sugarcane bagasse was obtained from the São Martinho Mill (Pradópolis, SP, Brazil). The cellulase complex Cellic® CTec3 was provided by Novozymes Latin América (Araucária, PR, Brazil). The industrial strain of *S. cerevisiae* (Thermossac® Dry) was obtained from Lallemand (Milwaukee, WI, USA).

2.2. Steam explosion

Steam explosion of sugarcane bagasse was carried out under conditions that were optimized previously (unpublished data). Pretreatment was performed with 1 kg of cane bagasse (50 wt% moisture content) at 195 °C for 7.5 min in a 10-L steam reactor. The resulting steam-exploded material (SEB) was washed twice with water for 30 min at room temperature under a 5 wt% total solids (TS). A fraction of the water-washed SEB was separated and characterized as described below. The remaining substrate was stored at 4 °C in vacuum-sealed bags until further use.

The water-washed SEB was also submitted to an alkaline extraction. The conditions used for this purpose were established by the optimization procedure described below. The resulting delignified substrate was referred to as sono-assisted alkali-washed steam-exploded bagasse (SA-AWSEB).

2.3. Alkaline delignification

The alkaline extractions were performed with milled native cane bagasse (1 mm particle size in average) in 500 mL Erlenmeyer flasks containing 5 wt% TS, using an agitation of 200 rpm during 30 min in an Ultronique Q5.9/37A ultrasonic bath. First, a preliminary study was carried out to evaluate the effect of ultrasound on alkali delignification. The alkali loading in these experiments was 0.5 g of NaOH g^{-1} of native bagasse (dry basis) and extraction was done at 80 °C for 30 min using 0 and 163 W of sonication power.

Once the beneficial effect of ultrasound was demonstrated, the sono-assisted alkaline delignification of cane bagasse was pre-optimized using a central composite design with two replicates at the center point for a total of 16 experiments (Table 1). The variables tested were temperature (°C), NaOH concentration (g g^{-1} TS) and sonication power (W). After delignification, the resulting substrates were filtered and washed with a fresh NaOH solution at the same concentration used before, followed by several water washing steps until a neutral pH was reached.

Small fractions of both alkali-washed (AWB) and sono-assisted alkali-washed (SA-AWB) cane bagasse were separated and characterized as described below. The remaining materials were stored at 4 °C in vacuum-sealed bags until further use.

2.4. Biomass chemical composition

Samples of sugarcane bagasse before and after pretreatment were dried at 105 °C for 2 h and Wiley-milled to pass a 40 mesh screen. The amount of extractives was only determined in native bagasse using the NREL/TP-510-42619 standard procedure [24]. The moisture content of all biomass samples was determined in an infrared balance (Shimadzu MOC63u) according to NREL/TP-510-42621 [25] and the ash content was measured as recommended by the NREL/TP-510-42622 standard procedure [26].

The biomass macromolecular components (carbohydrates and lignin) were determined on the basis of both NREL/TP-510-42618 [27] and NREL/TP-510-42617 [28] analytical methods. In these, acid-insoluble lignin is determined gravimetrically after acid hydrolysis while acid-soluble lignin is quantified by ultraviolet spectroscopy, respectively. The carbohydrates present in substrate acid hydrolysates were determined by high performance liquid chromatography (HPLC). Analyses were performed at 65 °C in an Agilent Hi-Plex H column with a suitable guard column. Elution was carried out with 5 mmol L^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1}. Quantification was performed by external calibration of the component sugars (cellobiose, glucose, xylose, and arabinose), acetic acid and dehydration by-products (furfural and hydroxymethylfurfural).

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