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Cold alkaline extraction as a pretreatment for bioethanol production from eucalyptus, sugarcane bagasse and sugarcane straw

Danila Morais de Carvalho [a,*], Olena Sevastyanova [b,c], José Humberto de Queiroz [d], Jorge Luiz Colodette [a]

[a] *Pulp and Paper Laboratory, Department of Forestry Engineering, Federal University of Viçosa, Av. P.H. Rolfs, s/n, Campus, 36570-900 Viçosa, Minas Gerais, Brazil*
[b] *Department of Fiber and Polymer Technology, KTH Royal Institute Technology, Teknikringen 56-58, SE-100 44 Stockholm, Sweden*
[c] *Wallenberg Wood Science Center, KTH Royal Institute of Technology, SE-100 44 Stockholm, Sweden*
[d] *Department of Biochemistry, Federal University of Viçosa, Av. P.H. Rolfs, s/n, Campus, 36570-900 Viçosa, Minas Gerais, Brazil*

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ABSTRACT

Optimal conditions for the cold alkaline extraction (CAE) pretreatment of eucalyptus, sugarcane bagasse and sugarcane straw are proposed in view of their subsequent bioconversion into ethanol through the semi-simultaneous saccharification and fermentation (SSSF) process (with presaccharification followed by simultaneous saccharification and fermentation, or SSF). The optimum conditions, which are identified based on an experiment with a factorial central composite design, resulted in the removal of 46%, 52% and 61% of the xylan and 15%, 37% and 45% of the lignin for eucalyptus, bagasse and straw, respectively. The formation of pseudo-extractives was observed during the CAE of eucalyptus. Despite the similar glucose concentration and yield for all biomasses after 12 h of presaccharification, the highest yield (0.065 $g_{ethanol}/g_{biomass}$), concentrations (5.74 g L^{-1}) and volumetric productivity for ethanol (0.57 g L^{-1} h^{-1}) were observed for the sugarcane straw. This finding was most likely related to the improved accessibility of cellulose that resulted from the removal of the largest amount of xylan and lignin.

1. Introduction

The global demand for energy continues to increase rapidly and exceeds the growth in the total energy supply [1]. At present, the primary focus is on the transformation of traditional energy production methods to more sustainable and environmentally friendly processes, in particular, the replacement of certain fossil-derived fuels by renewable resources [2–5].

Bioethanol is definitely the most-consumed biofuel worldwide, and it is used either in neat form or as an additive to gasoline [6]. Lignocellulosic biomass (e.g., wood, non-food and short-rotation herbaceous crops) is currently considered the best feedstock option for bioethanol production because of its relatively cheap and abundant production, which results in many economic, environmental and energy security advantages [2,7,8]. Bioethanol production from non-food sources such as sugarcane bagasse, bagasse pith, sugarcane straw, eucalyptus, corn stover, corn straw, coconut wastes, cactus and sweet sorghum has been studied by several researchers [9–18]. Brazil has great potential as a supplier of such non-food sources. Sugarcane is one of the country's primary agricultural crops and generates a large amount of wastes from both bagasse and straw. In fact, the forecast for the 2015/16 harvest is that approximately 92 million tons of bagasse and 92 million tons of straw will be generated [3,19]. Brazil is also an important producer of fast-growing wood, and the most widely cultivated species is *Eucalyptus*. Both eucalyptus wood and sugarcane wastes are suitable feedstocks for bioethanol production [15,20].

There are, however, a number of challenges that need to be overcome to make bioethanol production economically feasible. One is the recalcitrance of cellulose to enzymatic degradation, which is related to the chemical composition and morphology of any type of biomass [21]. Another is the need for improved methods for the saccharification and fermentation of sugar molecules into ethanol; the current methods are inefficient and expensive, and they could mature and improve [12,22].

Typically, some sort of chemical pretreatment is required prior to enzymatic hydrolysis to improve the accessibility and digestibility of cellulose that is present in lignocellulosic biomass [23]. Frequently, hydrothermal, acid and alkaline pretreatments are performed at high temperatures and pressures for biomass preparation [2]. Alkaline pretreatment is a promising process for the chemical transformation of biomass, and it results primarily in delignification when it is performed at high temperatures;

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* Corresponding author.
E-mail address: carvalho.danila@gmail.com (D.M. Carvalho).

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however, the removal of a significant amount of hemicellulose and the swelling of cellulose are also observed. These processes lead to improvements in the enzymatic hydrolysis of cellulose [24–26].

One variation of alkaline pretreatment is cold alkaline extraction (CAE), which is usually used for dissolving pulp [27], but few studies have explored other industrial segments, such as ethanol production. CAE is generally conducted at room temperature, which permits the use of simple, unpressured reactors and reduces power consumption. CAE promotes the fractionation of the chemical constituents of biomass into alkali-soluble lignin, hemicelluloses and other residues with fewer chemical and physical changes [28–30]. During CAE, the hemicellulose is removed from biomasses into liquor in polymeric form (i.e., without substantial degradation), which allows this biopolymer to be used for the production of new materials, such as biofilms [31,32]. After CAE, hemicellulose-depleted biomass is also lignin-depleted because of the substantial removal of the lignin that is chemically bonded to hemicelluloses (in the form of lignin-carbohydrates complexes). Biomass with these chemical characteristics is suitable for ethanol production because of its low concentration of potential enzymatic inhibitors, i.e., xylan and lignin-carbohydrate complexes [33].

Because of its double action (hemicellulose removal and lignin removal) and the relatively simple instrumental and processing conditions (a simple, unpressured reactor and room temperature), CAE seems to be a potential process for pretreating biomasses for ethanol production, but only a small amount of information about that combination is available in the literature. In addition, optimized conditions for CAE (e.g., the temperature, reaction time, alkaline charge, liquor/biomass ratio) vary depending on the biomass type, which requires optimization conditions for each biomass to improve ethanol production. In general, wood biomasses are more resistant to CAE than grass biomasses; thus, CAE alone is not suitable for extracting sugars from wood, resulting in low xylan extraction yields [28,34]. Additional measures, such as the use of milling to reduce the size of biomass, have been used to intensify the performance of pretreatments and enzymatic hydrolysis [17] and may provide a way to improve the efficiency of alkaline pretreatment for wood biomasses.

After pretreatments, the biomass is usually subjected to enzymatic hydrolysis. This process is also called saccharification, in which enzymes, such as cellulases, are used to promote the hydrolysis of cellulose chain-generating cellobiose and the hydrolysis of the cellobiose to glucose simultaneously. The sugars that are released during the saccharification steps are then converted to biofuels during the fermentation step [22].

Saccharomyces cerevisiae is a facultative anaerobic yeast, and it is typically used for fermentation in bioethanol production. Although it is able to convert C6 sugars to ethanol, it is not efficient at the conversion of C5 sugars derived from hemicelluloses [1].

One problem with regards to the saccharification process is the inhibition of enzymes that is caused by the saccharification products themselves (glucose and cellobiose in high concentrations). To avoid this inhibition, the simultaneous saccharification and fermentation (SSF) process for ethanol production has been investigated as a substitution for the traditionally separate hydrolysis and fermentation (SHF) processes [10,12,13,15,35]. During SSF, the enzymes (used for saccharification) and yeast (used for fermentation) are placed in the same reactor and glucose is converted to ethanol as soon as it is released [1]. In addition to suppressing this inhibition, SSF also results in a smaller demand for enzymes and a higher volumetric productivity of ethanol, in comparison with SHF [10,13]. Typically, enzymes require higher temperatures than yeasts can withstand [15]. Therefore, several recent studies have been performed with thermotolerant yeast strains, and promising results have been presented for strains such as *Kluyveromyces fragilis* NCIM 3358 [16], *Kluyveromyces marxianus* CECT 10875 [9] and *S. cerevisiae* LBM-1 [15]. Researchers have suggested the use of a presaccharification step prior to SSF to provide glucose in large enough concentrations to initiate yeast action at the beginning of the SSF process. This process, which was recently denoted as semi-simultaneous saccharification and fermentation (SSSF), can result in an increased ethanol yield and shorter processing time as well as higher cellulose-to-ethanol conversion than that with SSF alone (i.e., without the presaccharification step) [10,13,15,35,36].

In the present study, the CAE pretreatment for eucalyptus, sugarcane bagasse and sugarcane straw was optimized by using an experiment with a factorial central composite design that generated second-order polynomial models based on independent variables. The objectives were as follows: (i) to identify the optimal conditions (temperature, reaction time and NaOH concentration) for CAE in eucalyptus, sugarcane bagasse and sugarcane straw to improve xylose removal from biomasses; and (ii) to evaluate the efficiency of bioethanol production from the pretreated biomasses through a semi-simultaneous saccharification and fermentation (SSSF) process (i.e., presaccharification followed by simultaneous saccharification and fermentation). To the best of our knowledge, this type of approach (i.e., the optimization of CAE conditions for biomass preparation combined with the SSSF process to assess the ethanol yield from biomasses) has not been reported before. By using mathematical models such as those employed in the present study, however, various pretreatment technologies for the bioconversion of cellulosic biomass into transportation fuels can be assessed in a much faster and more efficient way.

2. Material and methods

2.1. Materials

Eucalyptus, sugarcane bagasse and sugarcane straw were used. Wood chips from a 7-year old clonal hybrid of eucalyptus (*Eucalyptus urophylla* × *Eucalyptus grandis*) were supplied by a pulp company and fragmented in a hammer mill (10 mm diameter). Bagasse and five-month-aged straw (cultivar RB867515) were supplied by the Center of Sugarcane Experimentation (Oratórios, Minas Gerais State, Brazil) after chipping (bagasse and straw) and juice removal (bagasse) into particles with a 10 mm diameter. These biomasses were stored in airtight plastic bags at room temperature prior to use, and their moisture contents were determined according to TAPPI T 264 cm-07 [37]. The following chemicals were used: sodium hydroxide lentils (analytical grade) (Merck Millipore, Germany), acetic acid (glacial) (Merck Millipore, Germany), a commercial cellulase preparation (Celluclast 1.5 L) (Sigma-Aldrich, Brazil) and a *S. cerevisiae* LBM-1 thermotolerant yeast strain that was isolated from fermentation vats in Brazil.

2.2. Methods

2.2.1. Cold alkaline extraction and washing

The CAEs were performed in a water bath with a controlled temperature. A 50-g biomass was treated with 500 mL of NaOH in a 1 L beaker. The initial liquor:biomass ratio was 10:1 (dry weight basis). The tests were performed under the following conditions: temperature (20, 30 and 40 °C), reaction time (10, 35 and 60 min) and NaOH concentration (70, 90 and 110 g L^{-1}). Treatments were conducted with periodic agitation with a glass rod. After the treatments, each sample was washed twice with 1 L of distilled water and filtered through a Buchner funnel containing a cloth as a filtering device. The pH of the treated biomass was neutralized to 6–7 with 2 N acetic acid. The treated biomass was dried to a constant weight at room temperature (23 ± 1 °C and 50 ± 2%