Renewable Energy 87 (2016) 607–617

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Physical–chemical–morphological characterization of the whole sugarcane lignocellulosic biomass used for 2G ethanol production by spectroscopy and microscopy techniques

Sandra C. Pereira [a], Larissa Maehara [a, b], Cristina M.M. Machado [c], Cristiane S. Farinas [a, b, *]

[a] Embrapa Instrumentation, Rua XV de Novembro 1452, 13560-970, São Carlos, SP, Brazil
[b] Graduate Program of Chemical Engineering, Federal University of São Carlos, Rodovia Washington Luiz, Km 235, 13565-905, São Carlos, SP, Brazil
[c] Embrapa Agroenergy, Parque Estação Biológica s/no, 70770-901, Brasília, DF, Brazil

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form
15 September 2015
Accepted 27 October 2015
Available online xxx

Keywords:
Sugarcane lignocellulosic biomass
Cellulosic ethanol
Compositional analysis
Sugarcane straw
Sugarcane bagasse
Sugarcane tops

ABSTRACT

The natural recalcitrance of sugarcane lignocellulosic biomass remains a challenge for second generation (2G) ethanol production. Here, the physical–chemical–morphological characteristics of the whole sugarcane lignocellulosic biomass (including bagasse, straw, and tops) from commercial sugarcane varieties were evaluated before and after dilute sulfuric acid pretreatment, in order to help predict the behaviors of these materials during the 2G ethanol process. Analyses using NMR, FTIR, XRD, and SEM showed that the properties of the sugarcane varieties evaluated here were very similar. The crystallinity index values calculated from the XRD results were also similar for the different residue fractions, and were higher after pretreatment due to the removal of hemicellulose. The lignin and crystalline cellulose FTIR absorption bands were most intense for bagasse, followed by straw and tops. NMR analysis identified the presence of skeletal aromatic and methoxyl groups, attributed to the lignin structure, with the intensity of the signals following the order: bagasse > straw > tops. SEM images showed that structural disruption followed the order: tops > straw > bagasse. The spectral and morphological differences helped to elucidate the characteristics that made the bagasse fraction of the sugarcane residue less susceptible to enzymatic saccharification. Differences between the spectra for straw and tops indicated that the straw was less easily digested by enzymatic action, as also indicated by the morphological analysis. The results demonstrate that the combined use of spectroscopy and microscopy techniques can contribute to understanding the behavior of different biomasses intended to be used for 2G ethanol production.

1. Introduction

Second generation (2G) ethanol production using lignocellulosic biomass offers an attractive means of improving productivity in the biofuels sector. In this process, the enzymatic hydrolysis is still considered one of the most challenging steps, and its efficiency is closely related to the performance achieved in the biomass pretreatment step. Understanding of the physical–chemical–morphological features of the lignocellulosic biomasses and elucidation of the components and mechanisms influencing their recalcitrance are needed for the design of suitable processes. However, traditional methods of compositional analysis in terms of the contents of cellulose, hemicellulose, and lignin are not sufficient to understand the complex multi-scale structures of the lignocellulosic materials [1–3]. Moreover, most conventional methods used to determine the composition of lignocellulosic materials are lengthy and laborious, requiring harsh reagents and generating large amounts of waste [4]. For these reasons, there is increasing use of faster techniques that enable more comprehensive and accurate characterization, together with the development of effective screening tools that can be applied to different types of lignocellulosic biomass. Instrumental methods that have been used for this purpose include nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction spectrometry (XRD), and scanning electron microscopy (SEM), which are capable of generating comprehensive qualitative and quantitative data [5–10].

Spectroscopy and microscopy techniques have been used to compare different lignocellulosic materials in terms of their

* Corresponding author. Embrapa Instrumentation, Rua XV de Novembro 1452, 13560-970, São Carlos, SP, Brazil.
E-mail address: cristiane.farinas@embrapa.br (C.S. Farinas).

http://dx.doi.org/10.1016/j.renene.2015.10.054
0960-1481/

potential for bioconversion into ethanol. For instance, Li et al. [11] explored the susceptibility to enzymatic hydrolysis of celluloses from five different biomasses, using XRD to determine the crystallinity index (CrI, %). Duan et al. [12] evaluated the effect of composition and structure of different poplar lines (transgenic and wild type) on the production of bioethanol, using FTIR to determine the lateral order index and total crystallinity index, and SEM for analysis of morphological features. This enabled better understanding of the results of enzymatic hydrolysis and distinction among the raw materials employed. Similarly, Lima et al. [13] were able to demonstrate the potential of novel Brazilian sources of biomass for bioethanol production. Structural differences and the contents of cellulose, hemicellulose, and lignin were evaluated using SEM and solid-state ^{13}C NMR.

Techniques including NMR, FTIR, XRD, and SEM have been widely employed to evaluate the structural changes caused by different pretreatments of sugarcane lignocellulosic biomass. The combined use of NMR and FTIR can provide detailed structural elucidation of the effects of pretreatment on this material, by observing the changes in the analytical signals associated with the main components (amorphous and crystalline cellulose, hemicellulose, and lignin). XRD can be used to estimate the crystallinity index, and SEM provides information on the morphology of the biomass. These techniques have been employed to investigate the effects on sugarcane bagasse structure of different pretreatments (sequential acid-base, oxalic acid fiber expansion, steam processing in the presence of CO_2 and SO_2, and delignification) [14–17]. Elsewhere, FTIR, XRD, and SEM analyses have been used to determine the structural changes in sugarcane residues caused by hydrothermal, dilute acid, and sequential hydrothermal-delignification pretreatments [18–20]. Sindhu et al. [2,21] performed a physical–chemical characterization of sugarcane tops pretreated with dilute acid and alkali in order to optimize enzymatic saccharification for bioethanol production.

In a previous study, we undertook a systematic comparison of the use of the whole residual lignocellulosic biomass (including bagasse, straw, and tops) from four commercial sugarcane varieties (SP79-1011, RB867515, SP81-3250, and RB92579) for the production of 2G ethanol [22]. The parameters assessed were chemical composition, susceptibility to saccharification, and fermentability, considering the responses of each sugarcane residue in the different steps of the process (dilute acid pretreatment, enzymatic hydrolysis, and alcoholic fermentation). No significant differences among the varieties of sugarcane were observed, but the residue types showed significantly different responses to the process conditions [22]. Therefore, in order to understand and elucidate the different responses of the sugarcane residue fractions (bagasse, straw, and tops) in the 2G ethanol production process, the aim of the present study was to carry out a systematic physical–chemical–morphological characterization of the whole sugarcane lignocellulosic biomass, before and after the dilute sulfuric acid pretreatment step. The techniques employed included microscopy (SEM) and spectroscopy (NMR, FTIR, and XRD). It is worth noting that, to the best of our knowledge, the present work is the first to perform such a systematic study of the whole sugarcane lignocellulosic biomass (including bagasse, straw, and tops) from different varieties of sugarcane.

2. Materials and methods

2.1. Sugarcane lignocellulosic biomass

The three residue fractions that composed the whole sugarcane lignocellulosic biomass (bagasse, straw, and tops) were kindly provided by the Sumaúma mill (Marechal Deodoro, Brazil). These raw materials were obtained from the processing of four commercial varieties of sugarcane (SP79-1011, RB867515, SP81-3250, and RB92579, symbolically represented in this work by K, M, Q, and X, respectively). Prior to use, the materials were dried at 45 °C to a moisture content of less than 10%. The samples were then milled and sieved to granulometry smaller than 2 mm prior to storage at room temperature until used in the experiments. Details concerning the agronomic characteristics of these sugarcane varieties and their compositional analysis (in terms of the contents of cellulose, hemicellulose, lignin, and ash) are provided in our earlier work [22].

2.2. Dilute sulfuric acid pretreatment

The lignocellulosic sugarcane residues were submitted to a pretreatment in sulfuric acid solution (1.5%, w/w), at a solid to liquid ratio of 1:10, in an autoclave at 121 °C for 30 min. After returning to ambient temperature, the mixtures were filtered under vacuum to recover the solid fractions, with suitable disposal of the liquid fractions. The solids were washed with distilled water to remove the soluble components adhered to the surfaces. At the end of the procedure, the pretreated materials were dried in an oven at 45 °C until reaching moisture content below 10%, and then stored at room temperature for later use.

2.3. Physical–chemical–morphological analysis

The whole sugarcane lignocellulosic biomass (bagasse, straw, and tops) obtained from the processing of the four commercial varieties (K, M, Q, and X), before and after dilute acid pretreatment (H_2SO_4, 1.5% w/w, 1:10 solid:liquid ratio, 121 °C, 30 min), was examined by microscopy and spectroscopy techniques, as described below. No additional sample preparation was performed, except for coating of the specimens with a gold layer prior to the SEM analyses.

2.4. X-ray diffraction spectroscopy (XRD)

The inherent crystalline nature of the whole sugarcane lignocellulosic biomass (untreated and dilute acid pretreated) was studied using a Shimadzu LAB-X XRD-6000 X-ray diffractometer, with Cu-Kα radiation ($\lambda = 1.54$ Å) generated at a voltage of 30 kV and a current of 30 mA. The 2θ scan range was from 3° to 60°, with a scanning rate of 2°/min and a step size of 0.02°. For the purposes of comparison, the X-ray diffractograms were normalized by the intensity of the diffraction peak at $2\theta = 22°$.

The crystallinity index was determined by means of the empirical method described by Segal et al. [23], according to:

$$CrI = \frac{(I_{002} - I_{am})}{I_{002}} * 100, \quad (1)$$

where CrI represents the relative degree of crystallinity (%), I_{002} is the peak intensity of the 002 crystal plane at $2\theta = 22°$, and I_{am} is the peak intensity of the amorphous phase at $2\theta = 18°$.

2.5. Fourier transform infrared spectroscopy (FTIR)

Changes in the functional groups due to the dilute sulfuric acid pretreatment were evaluated by FTIR analysis. The spectra of the whole sugarcane lignocellulosic biomass (untreated and pretreated) were obtained with a Bruker Vertex 70 FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. During the analysis, the samples were pressed against the diamond crystal of the ATR device. The scanning range was from 4000 to