Industrial Crops and Products 86 (2016) 289–294

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Exploring biomass deconstruction by phase-contrast tomography

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ARTICLE INFO

Article history:
Received 2 December 2015
Received in revised form 21 March 2016
Accepted 3 April 2016
Available online 7 April 2016

Keywords:
Biomass
Surface area
Recalcitrance
Phase-contrast tomography
Synchrotron radiation

ABSTRACT

Alternative and renewable fuels derived from lignocellulosic biomass offer a potential alternative to conventional energy sources. Biorefinery feedstocks are usually pretreated with acid, alkali, or high temperature/pressure treatments before enzyme hydrolysis to yield glucose and other useful sugars for fermentation to ethanol. If plant cell walls are to be efficiently deconstructed by conversion technologies, then a detailed understanding of their structural organization in different length scales is required. Here, a novel imaging technique provided an opportunity to probe the deconstruction of the biomass structure by alkali pretreatments under mild operation condition. High-resolution phase-contrast tomography (PCT) provided unique aspects on the recalcitrance-related changes of physical structures spanning macro and micrometer length scales. This approach revealed that the thickness of the plant cell walls was considerable reduced on the whole particle. However, long cracks could be particularly observed crossing over the thin-walled parenchyma cells, making each the fibrovascular bundles totally or partially unattached and independent. Such particle fragmentation was responsible for increasing its external specific surface area about 10 times, while the total surface area of the inner porous structure only doubled in value. The PCT and imaging processing techniques can be applied to different processing routes for ethanol conversion, providing new insights into the underlying mechanisms of biomass deconstruction.

1. Introduction

As the extensive use of fossil fuels over the past few decades has led to concerns over their depletion and negative impact on the environment, the scientific community is exploring alternative renewable energy sources (Chundawat et al., 2011a,b; Himmel et al., 2007). Among those, the production of the second generation cellulosic ethanol from the abundant and inexpensive non-food plant biomass ("lignocellulosic biomass"), such as agricultural residues, has emerged as a promising technology (Chundawat et al., 2011a,b).

Nevertheless, lignocellulosic biomass is generally recalcitrant due to its lignin-hemicellulose complex structure, and in some cases the cellulose crystallinity (Chang and Holtzapple, 2000; Ciesielski et al., 2014; Ding et al., 2012; Zhang and Lynd, 2004). This recalcitrance hinders the enzymatic hydrolysis treatments required to convert biomass into liquid fuel. So far, many thermochemical pretreatments such as alkali and dilute acid methods have been developed to overcome the recalcitrance of native cell walls to enzymatic deconstruction. Most pretreatments depolymerize and/or partly solubilize hemicellulose and lignin, inducing an increase in the accessible surface area to enzymes (Kumar et al., 2009; Laureano-Perez et al., 2005). However, the aforementioned pretreatments technologies still suffer from high-processing costs and relative low sugar yields (Himmel et al., 2007; Singh et al., 2014).

Efficient conversion technologies and less recalcitrant substrates are two key requirements to the successful implementation of cellulosic biorefineries (Himmel et al., 2007). Though significant strides have been made in elucidating important questions in the area of recalcitrance and deconstruction of biomass, the structural modifications in the plant tissue and cellular organization following thermochemical pretreatments are still an outstanding issue. Identifying recalcitrant structures in biomass is necessary for further improvements of pretreatment strategies and selection of more amenable substrates to enzyme attack.

In-depth structural characterization of lignocellulosic materials faces the challenge of the multiscale features of plant cell wall recal-

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http://dx.doi.org/10.1016/j.indcrop.2016.04.011
0926-6690/

citrance (Chundawat et al., 2011a,b). For example, vascular bundles and pith tissues are evident within the same grass stem at a scale of 10^{-4} m, different cell types may be identified at a scale of 10^{-5} m, the primary and secondary walls of individual cells may be differentiated at the scale of $\sim 10^{-6}$ m, and the individual cellulose microfibers that make up these walls have dimensions in the order of $\sim 10^{-9}$ m (Himmel, 2012). Such structural characteristics, including the morphology of plant tissues and its deconstruction, have been mainly studied by conventional techniques such as scanning electron microscopy, which do not allow observation of the bulk of materials and provides only bidimensional topographical images.

In our previous work (Isaac et al., 2015), it was demonstrated that phase-contrast tomography (PCT) is a powerful technique for the advanced characterization of the tissue architecture in lignocellulosic biomass. The hierarchically structured tissues encompassing both macroscopic and microscopic three-dimensional (3D) features were clearly visible in the PCT images of *in natura* piassava fibers (Isaac et al., 2015; Fratzl and Weinkamer, 2007). The ability of PCT to image lignocellulosic materials was also presented in Refs. (e.g., Marulier et al., 2015, 2012; Viguié et al., 2011; Verboven et al., 2008). Imaging soft tissues and small density variations are possible due to the increase of sensitivity of up to a factor of 10^3 for weakly absorbing materials (Cloetens et al., 1999).

Here, the deconstruction of plant tissues is investigated by PCT, providing new and valuable information on the structural changes during pretreatments that is not accessible by other techniques. PCT and imaging processing techniques are used to provide quantitative data on the recalcitrance-related changes of physical structures spanning macro and micrometer length scales. We report the impact of alkali pretreatments under moderate condition on sugarcane bagasse particles, demonstrating the potentiality and limits of this approach in answering questions such as what structural features change to render pretreated biomass digestible.

2. Materials and methods

2.1. Biomass source and pretreatments

Crushed sugarcane bagasse stalks were provided by a sugar and ethanol refinery in Minas Gerais, Brazil. The bagasse was dried in an oven with temperature of 100 °C for 24 h to remove the moisture. Thereafter, stems were hand sectioned using a razor blade into thin slices (~10 mm) prior to the pretreatment. The same batch of sugarcane bagasse was treated in alkali solution (1% NaOH for 40 min at 120 °C), which represents a moderate pretreatment severity. The prepared raw and pretreated bagasse fragments were placed in sealed bags and stored in a desiccator at room temperature.

2.2. Imaging plant tissues by phase-contrast tomography

The tissue organization in the sugarcane bagasse particles was studied by PCT using synchrotron radiation. Measurements were performed at the imaging setup of the BAMline at the German storage ring BESSY (Berliner Elektronenspeicherring—Gesellschaft für Synchrotronstrahlung m.b.H.), operated by the HZB (Helmholtz Centre Berlin for Materials and Energy) (Görner et al., 2001). During sample rotation, 1,800 radiographic projection images were taken. The X-ray energy used for the measurements was 17 keV. A PCO 4000CCD camera with 4008×2672 pixels (14 bit) was used for the experiments. Each voxel represents dimensions of $0.438 \times 0.438 \times 0.438$ μm. Because of the restricted field of view of the CCD camera, bagasse particles with diameters of less than 1 mm were chosen for the tomographic inspections. A phase retrieval algorithm for tomographic reconstruction based on Paganin et al. (2002) was applied.

2.3. Image processing

The evaluation of tomographic volume was focused on the plant tissue architecture. Voxels were identified as belonging either to cell lumen (or, simply called pores) or plant cell walls by applying an intensity threshold based on their gray level. Information on the number of pores and their geometric properties was extracted from tomograms using the commercial FEI Avizo® software. Each individual pore is defined as a configuration of $1 \times 1 \times 1$ or more connected 'dark' voxels, i.e., voxels which share a common face, edge, or corner. For the quantitative analysis, pores with volume $\leq$8 voxels were supposed to result from noise and were neglected. Concerning the surface area, each individual pore boundary is constructed by triangles and the pore surface area is estimated. The internal surface area of the substrate is estimated by adding the surface areas of all pores computed. The external surface area is computed by subtracting the internal surface area from the total value, also provided by the software.

3. Results and discussion

The structural modifications of recalcitrant structures in sugarcane bagasse subjected to thermochemical pretreatments are evaluated. Since the alkali pretreatments are widely used and investigated methods (Kumar et al., 2009), they have been chosen for this investigation. Sodium hydroxide (1% w/v) pretreatment of substrate was carried out in an autoclave at 120 °C for 40 min. Analyses of the raw and pretreated particles, such as fiber appearance and physical features of the cell lumens and gross surface areas, are explored by means of phase-contrast tomography and image processing techniques.

3.1. Morphological changes after alkali pretreatment

The tomographic reconstruction of the raw sugarcane bagasse fibers is shown in Fig. 1, which a rigid and ordered cellular structure can be visualized. Tissue integrity is observed to a large extent, but the collapse of some cells is also evident. This may be result of the preceding drying process of fibers, which can lead in irreversible disruption of cell walls. Each voxel (volume element of the 3D data set) corresponds to dimensions of $0.438 \times 0.438 \times 0.438$ μm.

Moreover, the anatomical components of the bagasse fragments (fiber structure and pith) are easy to identify in the 3D view displayed in Fig. 1a and also in the cross section of the particle presented in Fig. 1b. These images reveal differences in cell type and size and also the cell lumens (here, also called simply pores). As typical, vascular bundles are surrounded by sclerenchymatous cells and embedded in the parenchyma (Ding et al., 2012). Part of the tomogram exhibits epidermis remains, indicating that the particle originates from the outermost part of sugarcane culm. Near the epidermis, vascular bundles are closely packed to one another. Away from epidermis, they are more scattered and surrounded by a large number of parenchyma cells.

Independently of the region that the vascular bundles are found in the particle cross section, the sclerenchyma cells exhibit thick walls of approximately 5 μm, containing cell lumens with diameters between 6 and 75 μm. Parenchyma cells have thinner cell walls and contain pores ranging from 25 and 60 μm in diameter.

The alkali pretreatment has a remarkable effect on the bagasse morphology (Fig. 2). The tissue architecture of the pretreated sample is practically unstructured. Long cracks could be observed crossing over the parenchyma tissue, making each of the vascular bundles and surrounding schlerenchyma cells unattached and independent. Such fragmentation of the particle increases the external surface area (fiber surface). The sclerenchyma is also