Bioresource Technology 110 (2012) 232–238

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Effects of hardwood structural and chemical characteristics on enzymatic hydrolysis for biofuel production

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ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form 14 January 2012
Accepted 17 January 2012
Available online 31 January 2012

Keywords:
Biofuels
Enzymatic hydrolysis
Hardwood
Lignocellulosic
S/G ratio

ABSTRACT

This study investigated the influence of various hardwood characteristics on enzymatic hydrolysis. Important hardwood species, including three *Eucalyptus* species, were comprehensively characterized using quantitative ^{13}C NMR, image analysis and fiber quality analysis. Hydrolysis efficiency from all the hardwoods was correlated to the wood chemical composition and lignin characteristics. Among the key wood components that control enzymatic hydrolysis efficiency, lignin content, enzyme adsorption on substrate and, the ratio of syringyl/guaiacyl (S/G) of the pretreated feedstock were identified as the most important. No wood morphological feature was found to have a significant influence on enzymatic conversion of the pretreated samples.

1. Introduction

The great availability of cellulosic polymers (chitin, cellulose, and, heteropolysaccharides) on the planet in the form of biomass gives them the prominent position of one of the most promising feedstock for bioconversion into ethanol fuel. However, the process is being challenged by the recalcitrance of the cellulosic structure which is demonstrated by its virtual immutability to almost any chemical and enzymatic hydrolysis attempts.

Nearly any chemical or biological attempt at biofuel production (ethanol) will dictate the necessity of a pretreatment stage. A pretreatment stage entails introducing energy (chemical, biological, mechanical, electrical, photolytic, etc.) that will make cell wall carbohydrates more accessible to enzymes and expedite the breakdown of the sugar polymers to monomeric units for fermentation. The main objective of the pretreatment step is to make the substrate more accessible to enzymatic hydrolysis to attain the highest saccharification yield (production of monomeric carbohydrate units). Pretreatment steps have a significant impact on the design and efficiency of the bioconversion process and the overall profitability. Pretreatments might account for 25–35% of the cost of converting lignocellulosic biomass to ethanol (Eggeman and Elander, 2005) in which feedstock composition and subsequent chemical reactivity will dictate the necessary pretreatment stage for conversion to ethanol.

From a practical perspective, it has been conjectured that the resistance of lignocellulosics to component separation/processing arises primarily from high lignin quantity and distribution, low lignin reactivity (low syringyl/guaiacyl (S/G) ratio), high cellulose crystallinity, accessible surface area, fiber dimensions, degree of polymerization and others (Mansfield et al., 1999; Mooney et al., 1998; Ohgren et al., 2007; Yu et al., 2011). Also, the presence of phenolic residues has been reported as one of the major barriers to efficient enzymatic hydrolysis of carbohydrates in lignocellulosic biomass (Martin and Akin, 1988; Ximenes et al., 2010; Zhu et al., 2008). Lignin acts like a physical barrier protecting cellulose from enzymes degradation which reduces sugar accessibility and monomeric conversion. Studies have demonstrated not only covalent bonds between lignin and all major polysaccharides, (arabinoglucuronoxylan, galactoglucomannan, glucomannan, pectins, and cellulose), but also cross-links among them (Li and Khraisheh, 2010; Lawoko et al., 2005) where phenyl glycosides, benzyl ethers and benzyl esters, have been suggested as the main types of lignin–carbohydrate bonds in wood (Balakshin et al., 2007).

Therefore, as the chemical composition of the wood is changed, it is expected that the pretreatment step will need to be modified to preserve carbohydrates while making the cell wall structure more accessible to enzymes.

Because there are few studies showing the influence of lignin structure on the efficiency of pretreatment stages and subsequent carbohydrate conversion (Chen and Dixon, 2007; Dien et al., 2006), the present study focuses on the influence of various hardwood species characteristics on enzymatic hydrolysis efficiency. Important hardwood species, including three *Eucalyptus* species, were

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doi:10.1016/j.biortech.2012.01.085

comprehensively characterized using quantitative ^{13}C NMR (for lignin), image analysis (for fibers), and fiber quality analysis. Enzymatic hydrolysis, using 20 FPU of enzyme/g of substrate, was performed on all kraft-pretreated samples. Hydrolysis efficiency from all of the hardwoods was correlated to wood composition and lignin characteristics.

2. Methods

2.1. Materials

Eucalyptus nitens, E. globulus, E. urograndis, sweet gum (*Liquidambar styraciflua*), red maple (*Acer rubrum*), red oak (*Quercusrubra*), red alder (*Alnusrubra*), cottonwood (*Populus trichocarpa*), acacia (*Acacia mangium*) wood samples were used to evaluate their enzymatic hydrolysis performance after kraft pretreatment.

2.2. Wood pretreatments

Kraft pretreatment of wood chips was performed at 150 °C. An M&K digester was filled with 150 g (dry weight) of samples including excess of white liquor (liquor:wood ratio of 10:1) with an active alkali charge of 40% with 25% sulfidity. Pretreatment times were set to be 20, 30, 45, and 60 min. After the desired reaction time, the whole apparatus was cooled by running cold water through the digester. The samples were removed from the digester and washed with deionized water until a neutral pH was reached. High alkaline charge was used to minimize the influence of the different species feature in alkali consumption and reactions.

2.3. Enzymatic hydrolysis

One gram (dry weight) of pretreated sample was subjected to enzyme hydrolysis. The various samples were placed into a 50 ml centrifuge tube with enough buffer solution (pH 4.8) to bring the consistency down to 5%. Three commercial enzymes that were used are as follows: cellulase (*Trichoderma reesei*, NS-50013), xylanase (NS-50014), and β-glucosidase (*Aspergillus niger*, NS-50010) were added to the samples at a dosage of 20 FPU (filter paper unit)/g of substrate (Lee et al., 2009). The tubes were placed in an incubator shaker (180 rpm) for 48 h at a temperature of 50 ± 2 °C.

After incubation was completed, the samples were filtered using medium porosity glass crucibles and the residue washed one time with buffer solution and two times with deionized water. The solutions were then combined for carbohydrate analysis. The residue was dried (105 °C) for weight loss evaluation and compositional analysis.

A clear understanding of the results obtained from enzymatic hydrolysis efficiency has been expressed using three criteria. The first, called saccharification efficiency (%), takes into account the amount of carbohydrates present on pulp that was enzymatically hydrolyzed. Therefore, this number is based on the hydrolyzed sugars in the carbohydrates present in pulp. The second (sugar recovery, %) takes into consideration the enzymatically converted carbohydrates, based on the total carbohydrate content found in the original sample. The last one (sugar recovery g/100 g wood) takes into consideration the carbohydrates and lignin lost during pretreatment, and expresses the data as enzymatically converted sugar relative to the starting amount of sample. In general, sugar recovery may be the most important number because it measures the amount of carbohydrates in wood that was recovered as monomeric sugar over the pretreatment and enzymatic hydrolysis stages. Thus, this will be the number used in most of the data interpretation.

2.4. Compositional and structural analyses

Carbohydrate composition was determined through acid hydrolysis. A 0.1 g sample was swollen with 1.5 mL of 72% H_2SO_4 at room temperature with occasional stirring for 2 h. The mixture was then diluted to 3% H_2SO_4 using deionized water, transferred to a vial, sealed, and heated to 120 °C for 1.5 h. The resulting suspension was filtered, and the filtrate analyzed for monomeric sugar content. Monosaccharides were analyzed on a Dionex IC-3000 chromatography system having fucose as internal standard (Lee et al., 2010).

Klason lignin and acid-soluble lignin content were determined using the extractive free wood meal/residue according to the method of Dence (1992). The sum of Klason lignin and acid-soluble lignin was reported as the total lignin content.

Structural analysis of original lignin was performed using milled wood lignin (MWL) according to the method of Bjorkman (1956). Cellulolytic enzyme lignin (CEL) was isolated from pretreated substrate following the method proposed by Chang et al. (1975). The lignin samples were analyzed via ^{13}C NMR, according to Capanema et al. (2005). The ^{13}C NMR spectra of the lignin preparations in DMSO-d6 were recorded on a Bruker AVANCE 300 MHz spectrometer at 300 K, using a 90° pulse width, a 1.4 s acquisition time, and a 1.7 s relaxation delay. Chromium (III) acetylacetonate (0.01 M) was added to the lignin solution to provide complete relaxation of all nuclei. A total of 20,000 scans were collected.

2.5. Substrate pore size, enzyme adsorption and elemental analyses

Pore size was interpreted by analysis of the accumulation of freezing bound water. Its analysis was performed by differential scanning calorimetry (DSC) Q100 (TA Instruments, New Castle, DE) (Park et al., 2006).

The amount of enzyme adsorbed on the substrate was evaluated by filtrate collection and subsequent protein determination using a UV spectrometer. One gram of sample was placed in a 50 ml vial and the consistency was brought down to 5% by the addition of buffer solution (pH 4.8). Then, the sample was placed into a 4 °C cold room, where the experiment was executed. A 20 FPU/g substrate cellulase enzyme charge (*T. reesei*, NS-50013) was added and small samples of filtrate were collected during a period of 2 h, at 0, 5, 15, 30, 60, and 120 min. The filtrate was filtered for solids removal and Bradford reagent (Sigma–aldrich) was added. UV analysis performed at 595 nm. UV absorption for the blank (without enzyme) was set to be 0.

Elemental analyses (C, H, and N) for protein content evaluation were performed using a Perkin-Elmer 2400 elemental analyzer by the Department of Soil Science NCSU, Raleigh, NC, USA.

2.6. Quantitative wood anatomy

Eight hardwood species were selected for quantitative anatomic evaluation. Four chips of each species were cut to obtain transverse microtomes, and a 1% aqueous safranin solution was used to enhance contrast of the sections. Next, the samples were washed with deionized water, and placed on a warm plate at 75 ± 5 °C to dry. Each section was fixed on a glass slide with Permount and anatomical properties were measured using a light microscope (Nikon E200), 3CCD color video camera (Sony DXC-390), and an Image-Pro Plus 4.5 software. From each slide, four 546 μm × 410 μm-sized images at 200× magnification and four 273 μm × 205 μm-sized images at 400× magnification were taken randomly at 640 × 480 pixels resolution. On the lower magnification images, vessel number, vessel lumen diameter (μm), and vessel lumen area fraction (%), were measured. Fiber lumen area fraction (%) and double cell wall (μm) were measured on the higher magnification images.