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Research paper

Chemical and structural factors influencing enzymatic saccharification of wood from aspen, birch and spruce

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ABSTRACT

The susceptibility of untreated and sulfuric-acid-pretreated aspen, birch, and spruce to analytical enzymatic saccharification was studied in relation to their chemical composition and physical-structural features. The analytical data collected covered the mass fractions of lignin, carbohydrates, and extractives, the release of acetic acid, formic acid, and uronic acids by acid and alkaline hydrolysis, crystallinity and crystallite size, syringyl: guaiacyl (S:G) ratio of lignin, cellulose accessibility, FTIR spectra, images from SEM and fluorescence microscopy, and susceptibility to enzymatic saccharification using enzyme mixtures with and without supplementary xylanase.In the absence of pretreatment the mass fraction yield of Glc on the original dry wood in the analytical enzymatic saccharification increased in the order birch (16 g kg^{-1}) < spruce (35 g kg^{-1}) < aspen (150 g kg^{-1}). After acid pretreatment, the order changed to spruce (170 g kg^{-1}) < aspen (290 g kg^{-1}), birch (290 g kg^{-1}). The relatively high recalcitrance of untreated birch was not possible to relate to mass fraction of lignin, S:G ratio, cellulose crystallinity, or mass fraction of acetyl, but rather to structural features, such as a more compact surface structure with high density and low cellulose accessibility. The relatively high sugar yields from both untreated and pretreated aspen suggest that aspen wood is well suited as feedstock for production of liquid biofuels and green chemicals in forest-based biorefineries.

1. Introduction

The aims of this investigation were to explore the susceptibility to enzymatic saccharification of wood from aspen, birch and spruce, three common tree species in the forests of Northern Europe [1], and to understand differences in recalcitrance to biochemical conversion for production of biofuels and chemicals. The susceptibility to enzymatic saccharification was studied both with and without pretreatment. Pretreatment is used to reduce the recalcitrance of the lignocellulose and facilitate enzymatic saccharification of cellulose. The most common approach is hydrothermal pretreatment under acidic conditions, which mainly targets hemicelluloses [2,3].

Hardwood, such as aspen and birch, and softwood, such as spruce, differ with regard to both chemical and structural features. For example, the lignin of hardwood consists of significant fractions of both guaiacyl (G) and syringyl (S) units, whereas softwood lignin consists almost exclusively of guaiacyl units [4]. Glucuronoxylan is the main hemicellulose of hardwood, whereas glucomannan is common in softwood [5]. The constituents of the wood also differ with regard to the spatial distribution across the cell wall. The fraction of cellulose and hemicelluloses increases from the middle lamella to the primary cell wall and further on to the secondary cell wall, whereas the fraction of lignin decreases in the same direction [6–8]. Hardwood has a greater variety of cell types than softwood, the structure of which is simpler and more homogeneous than that of hardwood [6].

Several factors are believed to contribute to the resistance of lignocellulosic feedstocks to enzymatic saccharification, but the main factors that govern recalcitrance of different species of lignocellulose are not fully understood. Previous studies have indicated that the chemical characteristics of the lignocellulose are important for the recalcitrance [3,9–11]. For instance, high cellulose crystallinity has usually been thought to increase the recalcitrance to enzymatic saccharification [12–14]. However, as hydrothermal pretreatment under acidic conditions increases the crystallinity of the biomass, the correlation between cellulose crystallinity and digestibility has been questioned [15]. Hemicelluloses and lignin block the access of enzymes to cellulose [3,16]. Lignin also increases recalcitrance by catalytically non-productive binding of enzymes [17,18]. Soluble degradation products from hemicelluloses and lignin may cause partial inhibition of cellulolytic enzymes [19,20]. G-rich lignin has been reported to cause higher recalcitrance than S-rich lignin [9–11]. Acetyl groups on xylan have been proposed to have a negative influence on catalytically

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productive binding of enzymes to cellulose [21]. Reduced cell wall acetylation has been linked experimentally with improved enzymatic saccharification of cellulose [22,23].

As different lignocellulosic materials and methods were used in different studies, it is difficult to compare results and arrive at a comprehensive view of the main causes of lignocellulose recalcitrance. Moreover, structural factors that might affect recalcitrance have received much less attention than chemical factors. In order to achieve a better understanding of the recalcitrance of wood, it is therefore valuable to compare the recalcitrance of different species of woody biomass and characterize the materials using different analytical techniques taking both chemical composition and structure into account. Pretreatment will affect both the structure and the chemical composition of the wood. Therefore we hypothesize that the main causes of recalcitrance will differ not only depending on the wood species but also depending on whether pretreatment is taken into account or not.

Pretreatment and analytical non-exhaustive enzymatic digestion of untreated and pretreated aspen, birch, and spruce was performed using a rapid, miniaturized procedure that permitted parallel processing of multiple samples and statistical evaluation of the results. The aim of the analytical saccharification of cellulose was to detect differences between wood samples rather than to produce the maximum amount of sugar. Enzymatic digestion was performed with and without supplementary xylanase to evaluate the role of xylan in recalcitrance. Pretreatment and saccharification processes were analyzed using high-performance anion-exchange chromatography (HPAEC) for quantification of monosaccharide sugars, uronic acids, and aliphatic acids. In addition to analysis of the chemical composition, the potential role played by physical-structural features including cellulose accessibility, compactness, and lignin distribution was investigated. Wood samples were analyzed using scanning electron microscopy (SEM), fluorescence microscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), and the Simons' stain method. A better understanding of the recalcitrance of wood provides a foundation for the development of more efficient conversion technologies and more competitive forest-based biorefineries.

2. Materials and methods

2.1. Materials

Wood from three tree species, hybrid aspen, silver birch, and Norway spruce, was acquired from Umeå (63.83 N, 20.25 E) and surrounding areas. Wood from hybrid aspen (*Populus tremula* L. × *tremuloides* Michx.) was kindly provided by the Umeå Plant Science Centre, Umeå, Sweden. Wood chips from silver birch (*Betula pendula* Roth) and Norway spruce [*Picea abies* (L.) H. Karst] were kindly provided by MoRe Research AB (Örnsköldsvik, Sweden) and SEKAB AB (Örnsköldsvik, Sweden), respectively.

Debarked and chipped wood was milled (A11 Basic mill, IKA, Staufen, Germany) and thereafter sieved using 100–500 μm sieves (Retsch Analytical AS 200, Retsch, Haan, Germany). The wood samples were then freeze-dried to a dry-matter content of 100%. The moisture content was measured by using an HG63 moisture analyzer (Mettler-Toledo, Greifensee, Switzerland).

2.2. Determination of the chemical composition of aspen, birch and spruce

Portions (3 g) of freeze-dried wood samples were extracted with 200 cm^3 of a 9:1 volume ratio of petroleum ether (Petroleum Benzene, Merck, Darmstadt, Germany) and acetone using a Soxhlet system (Büchi Extraction System B-811, Büchi, Flawil, Switzerland) with 15 extraction cycles (2 h) [24]. The extracted samples were then air-dried at room temperature for about 16 h until the mass was stable. The mass fractions of lignin and carbohydrates were determined essentially according to the NREL/TP-510-42618 method [25], except for the determination of monosaccharides, which was done using HPAEC (Section 2.9).

The mass fraction of acetyl was determined according to Gille et al. [26] with five times scale up using a total amount of 1.5 mg of woody material (freeze-dried and ground with bead mill) soaked in 500 mm^3 water. The polymer-bound acetate was released by adding 500 mm^3 of a 40 kg m^{-3} aqueous solution of sodium hydroxide and incubated for 1 h at 30 °C in a shaking incubator. Then, the samples were neutralized with 500 mm^3 of a 36.5 kg m^{-3} aqueous solution of hydrochloric acid and centrifuged for 10 min at 20 817 RCF. The supernatant containing acetic acid was analyzed by HPAEC (Section 2.9).

2.3. Dilute sulfuric acid pretreatment

Pretreatment was performed using dilute sulfuric acid and the conditions used for spruce were more severe than those used for the hardwood species, as softwood is more recalcitrant [2]. The dilute sulfuric acid pretreatment was carried out using a single-mode microwave system (Biotage Initiator 2.0, Biotage, Uppsala, Sweden). The reactions (triplicates of each wood sample) were performed in glass vials (0.5–2 cm^3 reaction size, Biotage) equipped with magnetic stirring bars (10 mm diameter, Biotage). The reaction temperature for aspen and birch was 165 °C and the time was 10 min (resulting in a combined severity factor of 2.2). For spruce, 180 °C and 5 min were used (combined severity factor 3.0). The total mass of the reaction mixture was always 1000 mg which included 50 mg freeze-dried wood sample. The mass fraction of sulfuric acid was 1% for aspen and birch and 4% for spruce. The pretreatment conditions were based upon a series of pilot experiments for each wood species, and the combined severity was calculated according to Chum et al. [27].

The reaction mixture was centrifuged for 15 min at 14 100 RCF. The pretreatment liquid was collected for determination of the yields of monosaccharides, acetic acid, and uronic acids. Prior to enzymatic digestion, the pretreated wood pellet was washed two times with 1 cm^3 ultra-pure water and one time with 1 cm^3 sodium citrate buffer (3.5 kg m^{-3} citric acid, 8.3 kg m^{-3} sodium citrate, pH 5.2) in preweighed 2 cm^3 safe-seal microcentrifuge tubes (Sarstedt, Nümbrecht, Germany).

2.4. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) analysis

Py-GC/MS was used to determine the S:G ratios of the wood species and the pretreated wood. The analysis was performed at the Plant Cell Wall and Carbohydrate Analytical Facility of the Umeå Plant Science Centre (UPSC) (Umeå, Sweden) according to the method described in Ref. [28].

2.5. FTIR analysis

FTIR analyses were performed at the Vibrational Spectroscopy Core Facility of the Chemical-Biological Centre (KBC) (Umeå, Sweden). The milled wood samples were ground together with potassium bromide (KBr, Spectrograde, Fisher Scientific, Waltham, MA). The spectra were obtained on a Bruker IFS 66v/S FTIR spectrometer with a standard Deuterated Triglycine Sulfate detector, and fitted with a diffuse reflectance accessory (Bruker Corporation, Billerica, MA). The background and the measurements spectra were recorded at 256 scans and 4 cm^{-1} resolution. Three subsamples were taken and scanned separately. Experiments were carried out in vacuum. The spectra were baseline-corrected and normalized to the 1510 cm^{-1} maximum.

2.6. XRD analysis

XRD was performed with an AXS d8 Advance X-ray diffractometer (Bruker, Germany) using Cu Kα-radiation (40 kV, 40 mA) with a line-