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Effects of residual lignin and heteropolysaccharides on the bioconversion of softwood lignocellulose nanofibrils obtained by SO_2–ethanol–water fractionation

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HIGHLIGHTS

- Nanofibrils (LCNF) were produced from softwood after SEW fractionation.
- The effect of lignin and hemicelluloses on LCNF bioconversion was elucidated.
- Enzyme binding and activity on LCNF was monitored *in situ* by QCM-D.
- Hydrolysis of SEW LCNF produce sugar yields higher than those of fibers.
- SEW-based biorefinery may integrate nanomaterial and biofuel production.

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ABSTRACT

The amount of residual lignin and hemicelluloses in softwood fibers was systematically varied by SO_2–ethanol–water fractionation for integrated biorefinery with nanomaterial and biofuel production. On the basis of their low energy demand in mechanical processing, the fibers were deconstructed to lignocellulose nanofibrils (LCNF) and used as substrate for bioconversion. The effect of LCNF composition on saccharification via multicomponent enzymes was investigated at different loadings. LCNF digestibility was compared with the enzyme activity measured with a quartz crystal microbalance. LCNF hydrolysis rate gradually decreased with lignin and hemicellulose concentration, both of which limited enzyme accessibility. Enzyme inhibition resulted from non-productive binding of proteins onto lignin. Near complete LCNF hydrolysis was achieved, even at high lignin and hemicellulose content. Sugar yields for LCNF were higher than those for precursor SEW fibers, highlighting the benefits of high surface area in LCNF.

1. Introduction

The plant cell wall is an important reservoir of high energy polymers in the form of polysaccharides including cellulose, heteropolysaccharides, and phenylpropanoid macromolecules (lignin). These polymers are the main constituents of lignocellulose, the most abundant renewable material on Earth. Cellulose represents approximately 40% of the dry weight of lignocellulosic biomass and consists of unbranched 1,4-β-D-glucan chains that associate into microfibrils by strong hydrogen bonding and van der Waals forces. Heteropolysaccharides such as hemicelluloses, contain a diverse group of molecules including glucose, xylose, mannose, galactose, arabinose, and 4-O-methylglucuronic acid units and make up the second largest carbohydrate component in the cell wall (approx. 20–30 wt.% on dry basis) (Ebringerová and Heinze, 2000). Lignin, an aromatic polymer derived from

Abbreviations: LCNF, lignocellulose nanofibrils; SEW, SO_2–ethanol–water; QCM-D, quartz crystal microbalance with dissipation.

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p-coumaryl, coniferyl and sinapyl alcohols, acts as glue that binds cellulose and hemicelluloses playing key roles to preserve the integrity and stiffness of the cell wall and to confer antimicrobial properties (Boerjan et al., 2003).

The bioconversion of lignocellulosic biomass represents an attractive and environmentally friendly alternative to sugar and bioethanol platforms (Sun and Cheng, 2002; van Dyk and Pletschke, 2012). Two critical steps in bioconversion involve biomass pre-treatment and enzymatic saccharification of cellulose and hemicelluloses. The pre-treatment is essential to break down the linkages between cellulose, hemicelluloses and lignin making the substrates more accessible and reducing its natural recalcitrance to enzymatic hydrolysis. After pre-treatment, the structural polysaccharides in biomass are hydrolyzed with cellulases and hemicellulases to produce reducing sugars including glucose monomers that are further fermented to desired end-products by microorganisms. In the hydrolysis of cellulose three main types of cellulases can be used: endo-1,4-β-glucanases, EC 3.2.1.4, exo-1,4-β-glucanases, EC 3.2.1.91 and EC 3.2.1.176 (cellobiohydrolase) and β-glucosidases, EC 3.2.1.21 (cellobiases). However, in contrast to cellulose, the hydrolysis of hemicelluloses requires a more diverse group of enzymes because of its heterogeneous composition. Some of the enzymes used to hydrolyze hemicelluloses include endo-xylanase, acetyl xylan esterase, β-xylosidase, endomannanase and β-mannosidase amongst others (van Dyk and Pletschke, 2012).

Despite recent advances in our understanding of the enzymatic saccharification of lignocellulose, enzymatic hydrolysis is still a bottleneck in bioconversion given by the high costs associated with enzyme use (Leu and Zhu, 2013). This is particularly problematic for softwoods comprising pine, spruce, Douglas fir and larch. These species are abundant biomass sources in the Northern hemisphere and are characterized by having high hexose concentration, high density and low ash content. These characteristics make these feedstock highly attractive for bioconversion (Zhu and Pan, 2010). However, softwoods are more recalcitrant than other lignocelluloses to enzymatic saccharification and usually require high enzyme loadings to achieve high sugar yields. This is mainly due to high retention of lignin and hemicelluloses during acidic and alkaline pretreatments, respectively. It has been also shown that most available pre-treatments have low efficiency of cellulose purification, which limits the exploitation of residual non-cellulosic compounds during enzymatic digestibility (Iakovlev and van Heiningen, 2012a). Thus, viable approaches in biorefineries should include fractionation processes that improve enzymatic accessibility of lignocellulosic biomass and simultaneously extract value of all its components by producing multiple valuable products (Bozell and Petersen, 2010). In addition, processes that offer opportunities for low-price high-scale and high-price low-scale are highly desirable.

In fact, a promising candidate in biorefinery platforms is the SO_2–ethanol–water (SEW) fractionation (Iakovlev and van Heiningen, 2012a). Through SEW process it is possible to obtain fibers with different relative amounts of cellulose, hemicelluloses and lignin from softwood, hardwood and annual plants while allowing almost full recovery of SO_2 and ethanol. The dissolved hemicelluloses can be used as a feedstock for further processing, while lignin can be obtained as two fractions: low-sulfonated "organosolv" (S/C9 ratio close to 0.1) and lignosulfonates (S/C9 ratio 0.25–0.3), both having potential markets (note that the S/C9 ratio for typical acid sulfite lignosulfonates is close to 0.5). SEW fibers can be used in the pulp and paper industry, as feedstock in bioconversion and also for the production of regenerated fibers and nanocellulose (Iakovlev et al., 2014). In this later case, lignocellulose nanofibrils (LCNF) is a nanomaterial with valuable properties when used in composites, coatings and nanopapers, organic–inorganic hybrids and bioconversion (Zimmermann et al., 2010; Zhang et al., 2013). LCNF consists of fibrils with length and diameter in the micro and nanometer scale, respectively, and is usually obtained by mechanical deconstruction of fibers (class II size reduction type) that increases the lignocellulose pore size and makes biomass more accessible to enzymatic hydrolysis (Leu and Zhu, 2013; Hoeger et al., 2013). LCNF has been produced and characterized from several types of wood and wheat straw fibers obtained by conventional pre-treatments (Zimmermann et al., 2010; Ferrer et al., 2012; Hoeger et al., 2013). However, to our knowledge there are no reports available on the characterization of LCNF from softwood SEW fibers. This limits our understanding on how different fractionation processes affect nanofibrillation, especially because pre-treatments typically modify differentially the chemical properties of lignocellulose (van Dyk and Pletschke, 2012).

Sensing techniques such as quartz crystal microbalance (QCM) are very useful to monitor *in situ* and in real time the binding and/or catalytic activity of cellulases on different lignocellulosic substrates (Ahola et al., 2008; Martin-Sampedro et al., 2012). Furthermore, if LCNFs with different cellulose, hemicelluloses and lignin contents are used in QCM experiments they can provide valuable information on the contribution of residual cell wall components on the enzymatic hydrolysis. Here, several SEW fractionation conditions were used to produce fibers with different composition as well as corresponding LCNFs. This allowed a fundamental study of the influence of lignin and hemicelluloses on the enzymatic hydrolysis of softwood LCNFs.

2. Methods

2.1. SEW fractionation

Air-dried Norway spruce wood chips (93% dry matter content) were screened using O45; //8; //6; //4 and //2 mm screens. The fractions from the //4 and //2 mm screens were combined and used for SEW fractionation according to Iakovlev et al. (2014). SEW processing conditions were selected to produce fibers with given cellulose, hemicelluloses and lignin content. The fractionation liquors were prepared by injecting gaseous SO_2 at 6.0% and 12%, by weight, into a cold mixture containing 55 vol.% ethanol and 45 vol.% deionized water. Ethanol grade ETAX A (96.1 v/v.%) was utilized. The chips (25 o.d. g) and the liquor (liquor-to-wood ratio of 6 L kg^{-1}) were placed in 220 mL bombs. The bombs were put into a silicon oil bath at 135 and 165 °C (±1 °C) for 22.5–160 min (Table 1) including a heat-up time of about 8–9 min. The fractionation intensity was represented by the H-factor which combines the effects of temperature and time (Iakovlev et al., 2014). At the end of the given period of time, the bombs were rapidly removed from the bath and put into cold water. After cooling, the spent liquor was separated from the fibers using a nylon washing bag. The obtained SEW fibers were first washed twice with 50 mL of 40 v/v.% ethanol–water solution at 60 °C and then twice with 500 mL deionized water at room temperature. Table 1 includes the conditions used in SEW reactions and the obtained SEW fibers, which are indicated by numerals "1" to "5" according to increased combined content of residual lignin and heteropolysaccharides.

2.2. Chemical characterization of SEW pulps and LCNF

The residual lignin and carbohydrate content in SEW fibers and LCNFs were analyzed by a two-step stage sulfuric acid hydrolysis followed by HPAEC-PAD analysis of solubilized sugars, according to NREL/TP-510-42618. The first stage of the acid hydrolysis was performed at 72 w/w% (acid-to-pulp ratio 10 mL g^{-1}, 30 °C, 60 min), and the second stage at 4 w/w% (acid-to-pulp ratio