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Determination of surface-accessible acidic hydroxyls and surface area of lignin by cationic dye adsorption

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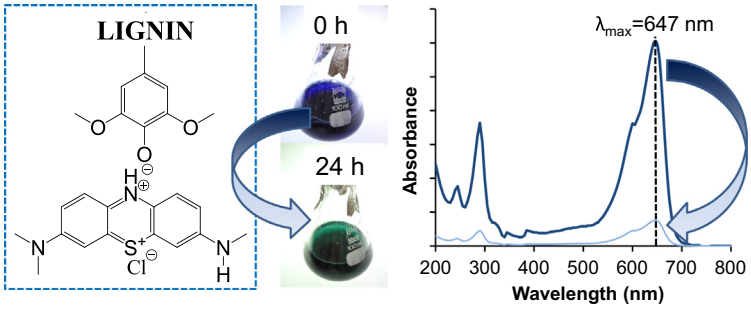
HIGHLIGHTS

- Surface-accessible acidic hydroxyls were determined by cationic dye adsorption.
- Lignin surface area (SA) was obtained based on the area covered by the dye Azure B.
- Wheat straw showed higher lignin SA than sugarcane bagasse or oat husks.
- Results were evaluated based on ^{31}P NMR analysis of isolated wheat straw lignins.
- Method was developed for characterization of lignin SA in solid state.

GRAPHICAL ABSTRACT



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ABSTRACT

A new colorimetric method for determining the surface-accessible acidic lignin hydroxyl groups in lignocellulose solid fractions was developed. The method is based on selective adsorption of Azure B, a basic dye, onto acidic hydroxyl groups of lignin. Selectivity of adsorption of Azure B on lignin was demonstrated using lignin and cellulose materials as adsorbents. Adsorption isotherms of Azure B on wheat straw (WS), sugarcane bagasse (SGB), oat husk, and isolated lignin materials were determined. The maximum adsorption capacities predicted by the Langmuir isotherms were used to calculate the amounts of surface-accessible acidic hydroxyl groups. WS contained 1.7-times more acidic hydroxyls (0.21 mmol/g) and higher surface area of lignin (84 m^2/g) than SGB or oat husk materials. Equations for determining the amount of surface-accessible acidic hydroxyls in solid fractions of the three plant materials by a single point measurement were developed. A method for high-throughput characterization of lignocellulosic materials is now available.

1. Introduction

Lignins are aromatic polymers synthesized mainly from three phenolic monomers called monolignols at proportions varying between plant species. The monolignols *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol contain zero, one, and two methoxy groups in the aromatic ring, and form *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) phenylpropanoid units of lignin, respectively (Grabber et al., 1997). Among other biological functions, lignin provides plants with protection against microbial degradation, as evidenced by negative linear correlation between lignin content and carbohydrate digestibility of forages and herbaceous plants (Jung, 1989; Sewalt et al., 1997a).

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Synthetic H, G, and S lignin polymers have similar inhibitory effects on cell wall degradability (Grabber et al., 1997), suggesting that inhibition might not be related to the number of methoxyl groups. Instead, phenolic hydroxyl groups of lignin preparations have appeared detrimental to cellulolytic enzymes (Sewalt et al., 1997b; Berlin et al., 2006). Most of the studies investigating the effect of lignin structure on enzymatic hydrolysis have been made using model compounds or isolated lignins. However, synthetic lignins might not adequately represent native lignin, as structure of isolated lignins depends on their origin in the plant tissues and the method used for their isolation (Sipponen et al., 2013).

Porosity increases the total surface area and heterogeneity of plant materials, and lignin in the pores may have a negative effect on hydrolysis of the plant material (Mooney et al., 1998). At least water conducting tissues are enriched in lignin (Laschimke, 1989), and it would thus be valuable to be able to measure non-degradatively the amount of surface-accessible lignin in plant solid materials. This could be achieved by determining acidic hydroxyls such as phenolic groups on surfaces. However, the known techniques are either degradative such as the chlorine dioxide titration, or, as in the case of reflectance UV-Raman spectroscopy, limited to measuring lignin on external surfaces only (Eshkiki et al., 2007; Lähdetie et al., 2009). Furthermore, the above mentioned methods have not been aimed at providing information of the surface area covered by lignin.

The objective of the current study was to develop a method for determining surface-accessible acidic hydroxyl groups of lignin in solid materials. The adapted approach is based on adsorption of Azure B onto lignin in aqueous suspension of the plant material. Like methylene blue, Azure B is a basic dye that carries a cationic charge in water, inducing binding to anionic acidic groups (Graham, 1955). Previously, Azure B has been used to study lignification of wood (Kutscha and Gray, 1972), but not in quantitative characterization of lignin. The current work was organized in three parts. First, specificity of adsorption of Azure B on isolated lignins in comparison to acetylated lignin and pure cellulose materials was elucidated. Second, the amount of surface-accessible acidic hydroxyl groups and the corresponding surface area of lignin were determined in wheat straw, sugarcane bagasse, and oat husk materials, based on the maximum equilibrium adsorption capacities predicted by the Langmuir isotherms. Finally, equations were developed for a single-point procedure for quantifying surface-accessible acidic hydroxyl groups in the three plant materials, varying for instance as a function of growth stage, pretreatment, or hydrolysis of associated carbohydrates.

2. Methods

2.1. Materials

Wheat straw (WS), sugarcane bagasse (SGB) was obtained from Danisco, and oat husk materials were used as test materials for comparative characterization of surface-accessible acidic hydroxyl groups. These agricultural residues were obtained from Finland (WS and oat husks) or from Brazil (SGB). Oat husks and WS were milled to pass a 1 mm sieve, suspended in cold water, wet-sieved and air dried. SGB was milled to pass a 1 mm sieve, and used as such. Wheat straw soda lignin (hereafter referred to as GreenValue lignin) originated from an industrial soda pulping process (Lora, 2008), and was purchased from GreenValue SA (Switzerland). Whatman I filter paper (Whatman, USA), was defibrillated by milling to pass a 1 mm sieve. Emcocel 50M microcrystalline cellulose (Penwest Pharmaceuticals, England) was used as such. Azure B was reagent grade and prepared by direct synthesis (Lot# MKBH6990V, CAS: 531-55-5, Aldrich, USA). Other chemicals used in this work were of analytical grade.

2.2. Preparation of extractive-free wheat straw and preparation of holocellulose

Extractive-free wheat straw (WS-EF) was prepared by Soxhlet extraction of WS (16.9 g) with distilled water, and then with ethanol (96%, v:v). WS-EF was air-dried and recovered at 65% yield. Preparation of holocellulose from WS-EF was carried out by sodium chlorite pulping in presence of acetic acid (Hallac et al., 2009). Briefly, 4.8 g of WS-EF was continuously stirred in 400 mL water containing 3.2 mL glacial acetic acid and 3.2 g sodium chlorite at 70 °C. After 2 h, similar amounts of acetic acid and sodium chlorite were supplemented and the treatment continued for 2 h. The solid fraction was recovered by filtration, washed with deionized water, and air-dried.

2.3. Preparation of WS lignin and acetylation of lignin

WS lignin was prepared from black liquor obtained from soda delignification of pre-extracted wheat straw (Pihlajaniemi et al., 2014). The black liquor (1500 g, 8.4% solids) was adjusted to pH 9.8, and after centrifugation the supernatant was acidified to pH 5 using 6 M sulfuric acid. The suspension was supplemented with 3 mL of commercial xylanase preparation GC 140 (Genencor), in order to remove insoluble xylan, and the continuously stirred 72 h enzymatic reaction was carried out at 50 °C. The solid and liquid phases were separated by centrifugation, and the solid fraction was washed three consecutive times with acidified water (pH 3, HCl) and lyophilized. Elemental analysis (CHN) showed that the obtained WS lignin contained 0.6% nitrogen as compared to 1.0% in GreenValue lignin. The two lignins were acetylated in pyridine:acetic anhydride (1:1, v:v) mixture, and purified following the literature procedure (Gosselink et al., 2004).

2.4. Compositional analysis

Lignin contents and carbohydrate compositions of the lignocellulosic materials were determined by the two-stage sulfuric acid hydrolysis procedure (Sluiter et al., 2010). Acid insoluble residue separated after the second stage hydrolysis by filtration on Whatman GF/F membrane was corrected for its ash content and termed Klason lignin. Ash content of the materials without any prior hydrolysis treatment was also determined by gravimetric method after ignition of the samples at 650 °C for 10 h. Carbohydrates in the lignin preparations (100 mg) were released by hydrolysis in 4% sulfuric acid (5 mL, 121 °C, 1 h). Monosaccharides were analyzed by high-performance liquid chromatography (HPLC) (Pihlajaniemi et al., 2014), the system comprising a liquid chromatography pump LC-6A, an autosampler SIL-20A, a column compartment CTO-20A, a refractive index detector RID-10A, and a System controller SCL-10A VP, all from Shimadzu (Japan). Analyses were carried out in duplicates and the mean values were calculated.

2.5. Analysis of lignin by ^{31}P NMR spectroscopy

Quantification of different hydroxyl groups in lignin was carried out using ^{31}P nuclear magnetic resonance (NMR) spectroscopy (Granata and Argyropoulos, 1995). The analysis was started by fully dissolving GreenValue lignin or WS lignin in 0.15 mL of dimethylformamide. Then, 0.1 mL of pyridine and 0.2 mL of internal standard endo-N-hydroxy-5-norbornene-2,3-dicarboximide (9.26 g L^{-1} or 8.97 g L^{-1}) in pyridine:deuterated chloroform solvent mixture (1.6:1, v:v) were added. Chromium(III) acetylacetonate (0.05 mL, 0.58 mg in the above solvent mixture) was added as a relaxation agent. Tetramethylphospholane (0.15 mL) was added slowly to start the phosphitylation reaction. Finally, 0.3 mL deuterated chloroform was added, and the solution analyzed with