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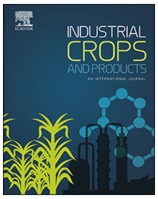

Experimental evidences for existence of varying moieties and functional groups in assorted crop waste derived organosolv lignins

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ABSTRACT

Isolation of organosolv lignins (ORGLs, water:ethanol, 180 °C, 1 h, 93 ± 5% mass balance) from various lignocellulosic materials like rice husk (RH), wheat straw (WS), bagasse (BG) & wood chips (WC) was done and experimental evidences are provided to perceive the differences in the structures of isolated lignins. To achieve this on bulk level, lignins were characterized by XRD, GPC, TGA & elemental analysis and on molecular level using UV–vis, ATR, 1D/2D HSQC NMR techniques. Besides isolated lignins, crop wastes and pulps were also characterized to divulge details on their properties. It was revealed that lignins have varying concentrations of sinapyl (*S*), coniferyl (*G*) and *p*-coumaryl alcohols (*H*) and tricin (T) moieties. While, Tricin (T) type lignin moieties are observed only in RH and WS derived lignins, RH derived lignin has higher concentration of side chains than other isolated lignins. Additionally, it is discovered that most lignins have *G* as main moiety.

1. Introduction

In recent times, employment of renewable feedstock, biomass for the synthesis of chemicals and fuels through bio-refinery concept is attracting lot of attention (Stöcker, 2008). In particular, exploitation of non-edible lignocellulosic biomass made up of C, H and O elements and obtainable in plenty (ca. 200 BMT/year) is more attractive (Bond et al., 2014; Maki-Arvela et al., 2011; Ragauskas et al., 2006).

Among the three major constitutes of lignocellulosic biomass; cellulose and hemicellulose have attracted considerable scrutiny towards the synthesis chemicals and fuels through catalytic and stoichiometric pathways (Bhaumik and Dhepe, 2016; Corma et al., 2007; Fukuoka and Dhepe, 2006). But, another major constituent, lignin is mainly utilized to produce heat and energy since it seizes highest calorific value compared to other two constituents and thus has not been given substantial attention for the synthesis of chemicals (Ragauskas et al., 2014). Researchers and policy makers have on numerous occasions voiced that until lignin is used to make valuable chemicals, the potential to expand the celluloses to ethanol process would not be justified (Huber et al., 2006; Li et al., 2015). Lignin is the only naturally occurring phenolic copolymer having 3-D amorphous structure and it consists of methoxy derivatives of phenylpropene moieties (sinapyl (*S*), coniferyl (*G*) and *p*-coumaryl alcohols (*H*), which are joined together through several linkages such as $\equiv$C—O—C$\equiv$ (60–70%) and $\equiv$C—C$\equiv$ (20–30%). Hence, this rich source of aromatic polymer is advocated to be used for making value enhancing aromatic compounds with lower molecular weight (Nimz, 1974). However, to achieve this, cleavage of ether and $\equiv$C—C$\equiv$ bonds is indispensable and it can be attained through various reactions such as hydrolysis, cracking, oxidation, hydrogenolysis etc. Yet, due to very rigid and complex structure of lignin this task becomes complicated to accomplish. Besides this, depending on the variety and age of plant & species, growth conditions such as moisture, period of year, temperature, soil condition etc. enhance the complexity of lignin structure and accordingly it becomes hard to depolymerise the same using generalized catalytic system. Furthermore, another considerable factor which affects the intricacy of lignin is the isolation methods used for its extraction from lignocellulosic biomass.

Separation of lignocellulosic components; cellulose, hemicellulose and lignin from each other is a preliminary step in a bio-refinery, wherein these components are separated from each other but, during this step invariably alterations in the their properties occur. It is observed that in case of cellulose and hemicelluloses major change occurs in terms of alternations in their degree of polymerization (DP) and crystallinity (cellulose), but mostly bonding and structure of sugars remain same. Nonetheless, in case of lignin due to various substituents present on aromatic ring and variety of bonds ($\equiv$C—O—C$\equiv$ e.g. β-O-4, 4-O-5, α-O-4 & $\equiv$C—C$\equiv$, e.g. β-1, β-5, β-β etc.), the changes become substantial and those are mostly unpredictable. Accordingly, substructural moieties of lignin like *p*-coumaryl alcohols (*H*), coniferyl alcohols (*G*) and sinapyl alcohols (*S*), their elemental composition (C, H &

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O), and molecular weight vary during separation processes. Again this deviation in composition is strongly dependent up on the method employed for its separation and origin of substrate. Numerous methods are known in the literature for the separation of lignin from lignocellulosic biomass such as, Kraft, hydrolysis, ammonia, enzymatic, ionic liquids etc. (Lange et al., 2013; Zakzeski et al., 2010). Typically, these methods use diversified conditions in respect to pH, pressure, temperature, reagents [alkalis (NaOH, CsOH, aq. NH_3, Na_2S, etc.), acids (H_2SO_4)], solvents, time etc. and those strongly affect the physico-chemical properties of extracted lignin (Section S1, Tables S1A & S1B, Supplementary material). In turn, these properties influence the product distribution up on depolymerisation of separated lignins (Kozliak et al., 2016). By understanding that with a change in separation method and substrate used, properties of lignin alter substantially, it becomes vital to study this aspect in detail. Nevertheless, the qualitative and quantitative assessment of several linkages and substituents present in lignin is an important challenge ahead of researchers. Due to the difficulties in comprehending the properties of separated lignins and establishing the substrate-product correlations in terms of substituents; largely researchers work with model compounds, which on smaller level replicate lignin structures for validating the depolymerisation prowess of catalysts. Nevertheless, these model compounds are devoid of any contaminations (Mg, Na, Ca, K, S, etc.) and are very simple molecules in comparison with actual lignin and thus it is certain that the catalytic systems developed with model compounds may not work in real scenario.

While many methods are known for separation of lignin from lignocelluloses, the most environmentally benign and less contaminating method is organosolv. It is established that the lignin obtained in organosolv method has many advantages such as; it is free of any metals, has higher solubility in many organic solvents and relatively has lower molecular weight (Lange et al., 2016; Zhang et al., 2016). Thus by understanding the benefits of organosolv method and acknowledging the impact of substrate on the properties of lignin (even if separated using similar method), it is crucial to understand the structures of lignin isolated from various crop waste substrates.

In view of above discussions, the aim of this study was to employ advanced characterization techniques (1D (^{1}H)/2D (^{13}C/^{1}H HSQC) NMR, ATR etc.) for scrutinizing the properties (lignin substructural moieties & functional groups) of diverse lignins derived from dissimilar type of lignocellulosic crop waste (rice husk (RH), bagasse (BG), wheat straw (WS) and wood chips (WC) using organosolv method.

2. Experimental

2.1. Materials

Different crop waste and wood chip samples were collected from different states of India; rice husk (RH) from Odisha, bagasse (BG) & wood chips (WC; from hard wood) from Maharashtra, and wheat straw (WS) from Uttar Pradesh. Before use, the crop waste and wood chip samples were sieved to obtain RH, WS, BG & WC with ca. 45 μm size. Ethanol (AR grade, 99.90%, Changshu Yangyuan Chemical, China), H_2SO_4 (98.0%, Loba Chemie, India), methanol (99.8%, Loba Chemie, India), 2, 5-dihydroxybenzoic acid (used for MALDI-TOF MS, > 99.5% Sigma-Aldrich, USA), acetonitrile (HPLC grade, 99.93%, Sigma Aldrich, USA), isopropyl alcohol (IPA, > 99.50%, Loba Chemie, India) and tetrahydrofuran (THF, HPLC grade, > 99.9%, Merck, India) were purchased and used without any further purification.

2.2. Analytical techniques

The physico-chemical characterisation of crop wastes & wood chips, recovered solid pulps and organosolv lignins (ORGLs) were carried out with several analytical instruments such as X-ray diffraction (XRD), scanning electron microscope along with energy dispersive x-ray spectroscopy (SEM-EDX), gel permeation chromatography (GPC), matrix assist lesser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), thermogravemetric analysis (TGA), elemental analysis, UV–vis spectroscopy, attenuated total reflection (ATR) and nuclear magnetic resonance spectroscopy (NMR). The details on the instruments and sample preparation are given in Section S2.a (Supplementary material).

2.3. Separation of lignin

Separation of lignin from RH, BG, WS & WC using organosolv method was done using following procedure. In a typical method, to a Teflon lined autoclave, 8.0 g sample was charged. To this, 60 mL distilled water & ethanol (1:1 *v/v*) was added and later 0.1 g H_2SO_4 was added. This charge was stirred at 180 °C for 1 h and later autoclave was cooled (Section S2.b, Supplementary material). Subsequently, solid and liquid parts were separated through whatman filter paper. The liquid contains dissolved lignin along with small quantities of sugars and soluble oligomers (product of hydrolysis of hemicelluloses and cellulose during acid treatment). The solid also known as pulp contains insoluble oligomers and unreacted cellulose and hemicelluloses along with wax. The solid (pulp) was kept for drying in an oven (55 ± 2 °C for 16 h). Further, the solid was dried under vacuum (−1.01 bar) at 90 ± 2 °C for 4 h. To the liquid part (containing soluble sugar/oligomers & lignin) 3 times of distilled water was added for precipitating out the fractioned hydrophobic lignin named as organosolv lignin (ORGL). Since lignin is soluble in ethanol and not in water, it was feasible to alter the water to ethanol ratio so that lignin could be precipitated out. The obtained ORGL was filtered through whatman filter paper & washed with plenty of distilled water to remove any acid contamination. Later this solid was also dried as described for pulp. For mass balance calculations please refer Section S2.c, Supplementary material.

3. Results and discussion

3.1. Physico-chemical analysis of ORGLs, pulps and crop wastes & wood chip

3.1.1. Bulk level understanding of ORGLs

The crop waste (RH, BG, WS) and wood chip (WC) samples were analysed for crystallinity by X-ray diffraction technique and the patterns are shown in Fig. 1 (Section S3.a, Supplementary material). As expected, two major peaks due to amorphous and crystalline nature of cellulose were observed at 2 Θ = 15.68° & 16.21° and 22.10°. The XRD patterns for pulp and isolated ORGL samples are shown in Fig. 1. Since, in ORGL samples only broad peak was observed, it indicates that the samples does not contain any impurity of cellulose and the lignin is amorphous in nature (Sarkanen, 1963; Tang et al., 2012). In case of pulp samples, peaks for cellulose were observed at 2θ = 15.68°, 16.21° and 22.10°. These results indicate that during extraction procedures, lignin is selectively extracted from crop waste materials (Wu et al., 2010). The observance of low intensity peaks in the BG sample (Fig. 1) compared to other samples, is must be because of higher content of hemicellulose in BG than any other samples (Bhaumik and Dhepe, 2014; Guilherme et al., 2015).

The SEM photographs of isolated lignin from various crop wastes showed similar type of spherical morphology. Typically, lignin particles with submicron size can be formed when lignin is heated above its glass transition temperature and also when oil in water type system is used. These lignin particles have in recent times gained lot of importance in various applications (antimicrobial, modification with metal ions etc.) due to their non-toxic nature. Here, it was possible to yield perfectly spherical individual and randomly distributed particles (droplets) of lignin with ~200–600 nm size (largely 200–300 nm) from RH, BG and WC crop wastes. However, lignin particles obtained from WS crop wastes showed agglomeration (lack of particle integrity) (Pu et al.,