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Membrane separation and characterisation of lignin and its derived products obtained by a mild ethanol organosolv treatment of rice straw

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ABSTRACT

An organosolv process using ethanol-water was optimized in order to recover high quality lignin from rice-straw previously pre-treated by autohydrolysis at 210 °C. The results showed a selective and appreciable removal of lignin under very mild conditions and the highest delignification yield occurred at 30 °C. The lignin extracts were characterised using capillary zone electrophoresis (CZE), size exclusion chromatography (SEC), Fourier transform infrared spectroscopy (FT-IR) and ^{31}P-NMR, and two-dimensional heteronuclear single quantum correlation NMR spectroscopy (2D-HSQC NMR), which enabled the identification of low molecular weight lignins with a syringyl/guaiacyl ratio of about 0.74 containing phenolic compounds with potential bioactive properties.

In order to separate the target compounds, membrane technology has been used and an enriched extract containing value-added phenolic compounds such as tricin, vanillin, ferulic acid and *p*-coumaric acid was obtained. High membrane efficiency (around 80%) was obtained for target compounds.

1. Introduction

Agricultural residues are considered interesting biomass feedstock for biorefineries due to their low cost and availability [1,2]. Their efficient utilisation within the biorefinery concept requires the selective fractionation of components to enable their separate exploitation for defined purposes [3]. Extensive research has been carried out for the separation of the three main structural components of biomass, hemicelluloses, cellulose and lignin, often in association with an initial removal of extractives [4].

In this framework, rice straw has been selectively fractionated by using a two-step process approach [5], namely, an hydrothermal treatment (autohydrolysis) is applied, by which most of the hemicelluloses are hydrolysed and can be recovered in the liquid phase as *xylo*-oligosaccharides and xylose, followed by a low intensity ethanol organosolv process to recover a liquid lignin fraction and a cellulose-enriched solid phase [5].

Both qualitative and quantitative composition of the organosolv lignin-derived stream may be influence by several operational parameters, most noteworthy, temperature; hence, this must be carefully studied. Characteristically, these streams contain many phenolic compounds, some of which are known for their potential bioactivity, but prior to their utilisation, its separation and purification is required. Typically, organosolv lignins can be first isolated by evaporation and/or freeze-drying of the solvent, but these processes have the disadvantage that they are not selective [4,6] and the recovered crude lignin must be further processed to obtain the relevant target compounds.

The application of membrane technology for processing the liquors from the delignification of a number of raw materials has been considered [7–9]. The use of ultrafiltration (UF) membranes, nanofiltration (NF) membranes or both has been applied mainly to remove non-lignin derived compounds from the extracts. For example, the combination of UF-NF was studied for the separation of hemicelluloses or other products, such as salts and monosaccharides, from black liquor [10,11]. The efficiency of various membranes for the fractionation and purification of hemicellulosic oligosaccharides was also demonstrated [11–13].

Membrane techniques can be particularly useful for the separation of lignin-derived compounds for added value applications such as

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health related novel materials (e.g. with anti-fungal and antibiotic activity), UV-absorption (e.g. cosmetics), antioxidants (i.e. radical scavenger), as well as stabilizer for food and feed purposes [14,15]. Lignin-derived compounds include monomeric phenolics such as vanillin and other bioactive phenolics *e.g.* with anti-carcinogenic and antibiotic activities [9,16–19]. These applications present much higher added-value when compared to the traditional use as energy source or in leather tanning, and have been already reviewed [14,20].

In the present study, the upgrading potential of hydrothermally treated rice straw was evaluated as a source of lignin-derived products. For that, a mild organosolv delignification was studied to selectively fractionate the lignin, and the solubilised lignin-derived compounds were recovered by using a solvent resistant NF membrane to separate target phenolic compounds such as tricin vanillin, ferulic acid and *p*-coumaric acid that were characterised by advanced analytical techniques.

2. Material and methods

2.1. Hydrothermal processing of rice straw

Milled rice straw was subjected to autohydrolysis in a 2 L stainless steel reactor (Parr Instruments Company, USA) as described before [5]. The raw material was mixed with water to a liquid-to-solid ratio of 10 (g water/g dry raw material) and the reactor heated to reach a final temperature of 210 °C. After cooling, the liquid and solid phases were separated by pressing; the liquid phase was filtered (Whatman filter paper n°.1) and the solid phase was washed, filtered again, dried at 40 °C, homogenised in a combined lot and used for delignification studies (2.2).

2.2. Organosolv delignification

The solids obtained from the hydrothermal treatments were subjected to organosolv delignification using an ethanol/water mixture of 60.5% (w/w) ethanol concentration, as described before [5]. Several conditions were tested to evaluate the effect of temperature (30–130 °C) and time (1–3 h) using a solid-liquid ratio of 1:10 (w/w). All experiments were carried out using Schott flasks in a thermostatic bath (30–70 °C) or in autoclave (90–130 °C). After reaction time elapsed, the flasks were rapidly cooled. The content was filtered and the liquid fractions were recovered and stored at 4 °C until further use. The sample obtained with 1 h delignification at 30 °C (OLRS30) was used for membrane separation. This sample and the sample obtained with 1 h delignification 110 °C (OLRS110) were also used for NMR, FT-IR and SEC characterisation, after vacuum evaporation and freeze drying. The solid phase was washed with twice the amount of water and then dried at 45 °C.

Delignification yields were determined as described before [5].

2.3. Membrane separation

Prior to the nanofiltration trials, the organosolv liquors extracted at 30 °C were filtered (Millpore 0.45 µm) and neutralized to pH 7 using $CaCO_3$, then filtered again (Millipore® 0,45 µm). Nanofiltration was carried out in a tailor-made stainless steel dead-end filtration cell using a semipermeable membrane (Evonix Industries, UK) (PuraMem™) with a *cut-off* of 280 Da. The membrane was washed with pure ethanol (99.9%) at 25 bar. The membrane preconditioning was performed using an ethanol/water mixture of 60.5% (w/w) at 45 bar, equal to the mixture used during the delignification process. In order to study the best separation conditions for the target compounds, a transmembrane pressure ranging from 15 to 40 bar was tested. The pressure was provided by compressed nitrogen gas and controlled by a regulator. All experiments were carried out at room temperature. The different permeates were analysed for total phenolics and composition profile by capillary zone electrophoresis (CZE). The membrane permeability was determined by calculating the slope of the linear regression between the sample permeation flux and the transmembrane pressure.

Membrane rejection was calculated by the expression:

$$R_i = \frac{1 - Ci,p}{Ci,f}$$

Where R_i is the apparent rejection of solute i (%), and Ci,p is the% of solute i in the permeate, and Ci,f is 100% of solute i feed to the membrane.

2.4. Analytical methods

2.4.1. Quantification of structural carbohydrates and lignin

The raw material and processed solids (after autohydrolysis and after delignification) were ground in a knife mill (particle size < 0.5 mm) and the moisture content was determined by oven-drying at 100 °C to constant weight. The ash content was determined at 550 °C using NREL/TP-510-42622 protocol [21]. The samples were hydrolysed with 72% (w/w) H_2SO_4 followed by hydrolysis with 4% (w/w) H_2SO_4 for determination of glucan, xylan, arabinan and acetyl groups. The acid insoluble residue was considered as Klason lignin, after correction for ash. Acid soluble lignin was determined as described in [21]. Monosaccharides (glucose, xylose, arabinose) and acetic acid were analysed by HPLC using an Aminex HPX-87H column (Bio-Rad, USA) in an Agilent Chromatographer, equipped with a diode array detector (DAD) and a refractive index detector (RI) as described before [5].

2.4.2. Identification and quantification of phenolic compounds

Total phenolic compounds were determined by the Folin-Ciocalteu colorimetric method and their concentration expressed as gallic acid equivalents (GAE).

The phenolic profile was obtained by CZE using an Agilent System (Waldbronn, Germany), with diode-array detector (DAD), ChemStation data software and a fused-silica uncoated i.d. 50 µm and 62/56 cm effective length, extended light path capillary also from Agilent. A 30 kV voltage was applied and injection was done at 50 mbar for 6 s. A 15 mM borate in 10% MeOH was used as electrolyte adjusted to pH 9.1 and temperature was maintained at 25 °C. The capillary was preconditioned between runs by flushing with 0.1 M NaOH (3 min) followed by buffer (3 min). Detection was at 200 and 280 nm and compounds were identified by electrophoretic comparison (migration times and UV spectra) with authentic standards.

2.4.3. Molecular weight characterisation by size exclusion chromatography (SEC)

The molar mass distribution of lignin samples was analysed by alkaline SEC using a TSK gel Toyopearl HW-55F column, 0.5 M NaOH as eluent, UV detection at 280 nm and calibration with sodium-polystyrene sulfonates, as described elsewhere [22]. Mp (peak molecular weight), Mn (number average molecular weight), Mw (weight-average molecular weight) and polydispersity (PD, Mw/Mn) were calculated.

The following technical lignins [23] were used for comparison: soda wheat straw lignin (Soda WS) and soda lignin from mixed Sarkanda grass and wheat straw (P1000) obtained from Greenvalue SA (Lausanne, Switzerland) as well as organosolv lignin from mixed hardwoods (Alcell) obtained from Repap Technologies Inc. (Valley Forge, USA).

2.4.4. FT-IR spectroscopy

Fourier Transform Infrared (FT-IR) spectra of the lignin samples were obtained on a Varian Scimitar 1000 FT-IR (USA) spectrometer equipped with a DTSG-detector and a PIKE MIRacle ATR and a diamond w/ZnSe lens single reflection plate. Spectra were collected in attenuated total reflectance (ATR) mode in the range 4000–650 cm^{-1} with a resolution of 4 cm^{-1} and with 128 co-added scans.

2.4.5. Phosphor NMR (^{31}P-NMR)

^{31}P-NMR spectra of lignin samples were recorded on a Bruker