Biomass and Bioenergy 117 (2018) 190–197

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

Evaluation of hardwood and softwood fractionation using autohydrolysis and ionic liquid microwave pretreatment

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ARTICLE INFO

Keywords:
Softwood
Hardwood
Autohydrolyis
Microwave ionic liquid
Confocal fluorescence microscopy
Biomass pretreatment

ABSTRACT

Differences in hardwood and softwood, elucidates their behaviour against pretreatments varies. In this work, microwave ionic liquid (IL) and autohydrolysis (AH) pretreatments were applied to *Eucalyptus globulus* (as a model of hardwood) and *Pinus radiata* (as a model of softwood). The comparison between hardwood and softwood of microwave ionic liquid (IL) and autohydrolysis (AH) were evaluated in terms of chemical composition of pretreated solids, liquid by streams composition (hemicellulose and lignin extraction) and, substrates enzymatic digestibility. Furthermore, micrographs using scanning electron microscopy (SEM) and confocal fluorescence microscopy supported results obtained. In this study, it has been demonstrated that autohydrolysis pretreatment effectiveness, through maximizing enzymatic digestibility, is opposite in hardwood (73 g glucan/100 g glucan introduced at severe conditions) and softwood (10 g/100 g glucan). IL pretreatment has been especially effective in softwood with higher digestibilities (78 g glucan/100 g glucan introduced) than those obtained in hardwood (68 g glucan/100 g glucan introduced). Confocal fluorescence microscopy images, together with SEM images have resulted to be a clarifying technique to explain enzymatic digestibility results. Final sugars yields after the whole process have shown that low solid yields recoveries obtained in AH treatments have considerably worsened final glucose production, mainly in softwood. IL microwave pretreatment have resulted in higher glucose yields in softwood than in hardwood.

1. Introduction

Despite recent advances, the biorefinery of lignocellulosic biomass is still a challenge [1]. There is a bottle-neck of the process in the conversion of complex carbohydrates to fermentable sugars [2]. Lignocellulose is a recalcitrance non-uniform three-dimensional structure, that requires pretreatment processes to deconstruct the linkages and disrupt the structure [3]. Pretreatment technologies can constitute up to 40% of the total processing costs of lignocellulosic biomass conversion [4].

Autohydrolysis (AH) has been described as an inexpensive, environmentally friendly and easy-handle process to selectively remove hemicellulose with low cellulose and lignin degradation [5,6]. AH only uses water as reactive, which results in the water autoionization towards acid hydronium ions (H_3O^+). Oligosaccharides obtained in the liquid phase are value added products used in food and pharmaceutical industries [7]. Ionic liquids (ILs) are effective biomass solvents that reduce recalcitrance, enabling deconstruction, and disruption of the lignin and hemicellulose network [8]. The non-flammability, high chemical and thermal stability, and negligible vapour pressure are some of the advantages against other pretreatment processes [9]. ILs also reduce cellulose crystallinity, increasing its accessibility and favouring high glucose conversions [10–12]. ILs are good microwave absorbers, enhancing biomass conversion processes and fastening the heating rate [13,14] However, the use of microwave must be assessed to avoid very severe conditions that may produce degradation [15].

Some studies have already been reported comparing softwoods and hardwoods pretreatments [16–19]. Pretreatments are, in general, more effective in hardwoods than in softwoods [19].

In this work, a comparison between hardwood and softwood pre-

Abbreviations: IL, Ionic liquid; [Emim][OAc], 1-ethyl-3-methylimidazolium acetate; AH, autohydrolysis; OS, oligosaccharides

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https://doi.org/10.1016/j.biombioe.2018.07.014
Received 20 November 2017; Received in revised form 4 June 2018; Accepted 20 July 2018
0961-9534/

treatment efficiency are performed. Autohydrolysis and IL microwave pretreatments at different severity conditions are carried out. Chemical compositions of solid and liquid fractions for each pretreatment were determined. Solids enzymatic hydrolysis digestibilities were evaluated, compared and, supported with morphological structures obtained from scanning electron microscopy and confocal fluorescence laser microscopy techniques, in each case. In this study, identical enzyme cocktails, loading levels, and analytical methods were employed in order to offer an overview of the sugars and by-products obtained.

2. Material and methods

2.1. Materials and reagents

The ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim][OAc], > 95%, Iolitec GmbH) was employed for wood dissolution. *Eucalyptus globulus* and *Pinus radiata* sawdust were provided by the National Institute for Agronomic Research (CIFOR-INIA). Organosolv lignin from *E. globulus* and *P. radiata* wood were used as reference materials, for UV/VIS measurements (explained below). Sulfuric acid was used to precipitate lignin from the mixture. The enzymatic cocktail Accellerase 1500®, containing 70 mg of protein/mL of dissolution was donated by Dupont Industrial BioSciences and used in the enzymatic hydrolysis step.

2.2. Experimental

2.2.1. Autohydrolysis (AH) pretreatment

AH pretreatments were developed in a 450 mL stainless steel pressure reactor (Parr Instrument Company, model 4567). The reactor was fitted with a four blade turbine impeller working at 150 rpm. Before pretreatment, wood chips were milled and sieved to obtain a particle size between 0.3 and 2 mm. Eucalyptus and pine wood sawdust were mixed with deionized water in a liquid:solid ratio of 8:1 and 10:1 (g water: g dry biomass), respectively [20,21]. Mild autohydrolysis were developed at 150 °C for 30 min for both woods (AH150E for eucalyptus wood and AH150P for pine wood); intermediate, at 175 °C for 30 and 60 min in the case of eucalyptus and pine wood (AH175E and AH175P); and severe autohydrolysis conditions were performed at 200 °C for 30 and 90 min (AH200E and AH200P). The AH severity factor (S_0) was calculated [22].

2.2.2. IL microwave pretreatment

Eucalyptus and pine were milled and sieved to obtain particles with sizes < 150 μm. Extractives were removed using acetone and water to avoid foam formation that may cause dissolution problems [23,24]. 0.8 g extractives free samples of eucalyptus and pine wood were mixed with 20 g of [Emim][OAc]. Samples were heated under microwave irradiation in a Berghof SpeedWave Four microwave oven, using a two-step programme detailed in a previous work [25]. Operation temperatures were 80 °C (IL80E for eucalyptus and IL80P for pine wood) and 120 °C (IL120E and IL120P) in a total time of 50 min.

Afterwards, 50 mL of deionized water was added to precipitate the wood dissolved in the process. The solution was stirred for 10 min in a water bath at 40 °C, and was subsequently filtered under a vacuum to obtain the pretreated wood. Pretreated samples were washed 5-fold with 70 mL of deionized water.

2.2.3. Enzymatic hydrolysis

The enzymatic hydrolysis was carried out in an orbital incubator at 150 rpm and at 50 °C. 1 % (w/w) pretreated wood (< 150 μm) was suspended in 50 mM citrate buffer (pH 5.0) containing 0.002% of sodium azide in a working volume of 8 mL. Accellerase 1500 enzymatic cocktail in a dosage of 0.25 mL/g glucan was added. Aliquots of 150 μL were periodically taken at 3, 6, 12, 24, 48 and 72 h, and centrifuged to stop the enzymatic reaction.

2.3. Analytical methods

2.3.1. Chemical characterization of biomass samples

Biomass compositions, before and after pretreatments, were determined according to the NREL/TP-510-42618 methodology adapted to small quantities of samples [26,27]. The acid-soluble lignin amount was determined using a Varian Cary 50 UV-VIS spectrometer at 205 and 240 nm, with an absorptivity of 110 and 12 $L\,g^{-1}\,cm^{-1}$ for eucalyptus and pine wood, respectively. Sugars in the hydrolysate were determined by HPLC, neutralizing with $CaCO_3$ and, filtering under 0.45 μm before the analysis, using a 300 × 7.8 mm Casbosep-CHO 682 column with Micro-Guard cartridges (BioRad, Life Science Group Hercules, Ca) at 80 °C, using water as mobile phase, and a flow rate of 0.4 mL/min.

2.3.2. Morphology of biomass samples

A Jeol JSM 6400 scanning electron microscope (SEM) was employed to observe the surfaces of untreated and pretreated samples. A Gold sputtering onto the sample surface was used to impart electrical conductivity. The operation voltage of the SEM was 20 kV. Analysis were developed in the technical facilities of the Spanish National Centre for Electron Microscopy.

A Leica SP-2 AOBS confocal laser microscope was used to visualize supramolecular structure changes. A laser at 405 nm was used to excite samples for fluorescence visualization. Wavelength emission ranges were 428–480 nm and 547–658 nm for hollocellulose and lignin (autofluorescent) respectively. β-1-4 polysaccharides linkages were dyed using 0.1% Calcofluor white stain [3]. Images were acquired at a step size of 2 μm and were combined into a z-axis max projection using Image-J software. Analysis were developed in the technical facilities of the Centre for Cytometry and Fluorescence Microscopy of the Complutense University of Madrid (UCM).

2.3.3. Chemical composition of autohydrolysis liquors

Monomeric sugars, organic acids and furans obtained from autohydrolysis liquors were directly measured by HPLC using a refractive index detector, according to the NREL/TP-510-42623 procedure [28]. The above mentioned operating conditions were used for sugars determination. Organic acids and furans were determined, using a Rezex ROA-Organic Acid H+ (8%) 300 × 7.8 mm column at 60 °C, with a mobile phase (0.005 M H_2SO_4) eluted at 0.6 mL/min.

2.3.4. Chemical composition of liquid by-stream obtained after microwave IL pretreatment

Recovered IL in the washing fractions was quantified by HPLC equipped with a UV detector measuring at 235 nm. The Eclipse Plus C18 4.6 × 100 mm column was operated using a mixture of acetonitrile/water 50/50 % (v/v) as mobile phase with a flow rate of 1 mL/min and at 30 °C.

The lignin content accumulated in the IL was analyzed by UV/VIS spectroscopy using a Varian Cary 50 scan UV/VIS spectrophotometer. A rotary evaporator was used to remove the water and recover the IL. Samples were diluted in 0.1 N NaOH and filtered, to measure the absorbance at 280 nm [29]. Water content in the recovered ionic liquid was determined using a thermobalance and mass was corrected. The total dissolved lignin concentration was obtained from the reference curve of eucalyptus and pine wood organosolv lignin samples. Reference samples used were prepared. The absorbance measurement of