



Hydrothermal pretreatment of several lignocellulosic mixtures containing wheat straw and two hardwood residues available in Southern Europe



Talita Silva-Fernandes^{a,b}, Luís Chorão Duarte^a, Florbela Carvalheiro^a, Maria Conceição Loureiro-Dias^b, César Fonseca^{a,*}, Francisco Gírio^a

^a Laboratório Nacional de Energia e Geologia, I.P. (LNEG), Unidade de Bioenergia, Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal

^b Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Linking Landscape, Environment, Agriculture and Food (LEAF), Tapada da Ajuda, 1349-017 Lisboa, Portugal

HIGHLIGHTS

- Autohydrolysis was an efficient pretreatment for mixtures of lignocellulosic materials.
- Autohydrolysis profile is similar for different feedstock mixtures.
- Sugar-based biorefineries can use mixtures of hardwoods and agricultural residues.
- Feedstock mixtures potentially mitigate biomass supply constrains in biorefineries.

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ABSTRACT

This work studied the processing of biomass mixtures containing three lignocellulosic materials largely available in Southern Europe, eucalyptus residues (ER), wheat straw (WS) and olive tree pruning (OP). The mixtures were chemically characterized, and their pretreatment, by autohydrolysis, evaluated within a severity factor ($\log R_0$) ranging from 1.73 up to 4.24. A simple modeling strategy was used to optimize the autohydrolysis conditions based on the chemical characterization of the liquid fraction. The solid fraction was characterized to quantify the polysaccharide and lignin content. The pretreatment conditions for maximal saccharides recovery in the liquid fraction were at a severity range ($\log R_0$) of 3.65–3.72, independently of the mixture tested, which suggests that autohydrolysis can effectively process mixtures of lignocellulosic materials for further biochemical conversion processes.

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1. Introduction

Many lignocellulosic residues, by-products and energy crops have been tested in laboratory and demonstration scales to be used as feedstock for biorefineries (Fava et al., 2015; FitzPatrick et al., 2010; Larsen et al., 2008; Requejo et al., 2012; Saadatmand et al., 2012). However, the limited biomass availability and supply can be considered one of the major problems restraining the global biorefinery deployment (Balat, 2011). The seasonal nature and annual variability of biomass supply may impair the use of many feedstocks due to logistic/economic reasons (Yue et al., 2014), namely high transportation and storage costs. Moreover, the volatility of biomass cost and the dependence of specific suppliers are

factors threatening the stability of a biorefinery (Gnansounou, 2010). The use of different biomass sources throughout the year may overcome these constrains, but different processing requirements may limit the success of a biorefinery.

An alternative that can minimize the problems related with biomass availability, seasonality, price volatility and storage, is the use of feedstock mixtures rather than a single raw material. Although some studies on mixtures are already available (Jensen et al., 2008; Martin et al., 2008; Thomsen and Hauggaard-Nielsen, 2008), the focus on technical evaluation of processing feedstock mixtures in the initial biomass pretreatment/fractionation stage is still scarce.

In this work, eucalyptus residues (ER), wheat straw (WS) and olive tree pruning (OP) were chosen as representative feedstock from Southern Europe, as they are widespread and significantly available in relatively concentrated regions. For instance, their

* Corresponding author. Tel.: +351 210 924 717.

E-mail address: cesar.fonseca@lneg.pt (C. Fonseca).

availability in the Iberian Peninsula (Portugal and Spain) by 2013 was estimated in 2.8×10^6 , 1.0×10^7 and 4.0×10^6 ton per year of ER, WS and OP (European Union, 2013; FAOSTAT, 2014; Kim and Dale, 2004; Romero-García et al., 2014). Furthermore, the seasonality of these materials is partially complementary, since ER is available throughout the year, WS is harvest in late spring/early summer, and OP is mainly available in winter and early spring. This further potentiates their use in a multi-feedstock biorefinery under continuous operation throughout the year, as also their storage costs are not expected to be significant as they have low moisture content, or can be easily dried.

These feedstocks are polysaccharide-rich materials, reaching 57% (ER), 68% (WS) and 62% (OP) of carbohydrate content (Silva-Fernandes et al., 2015). However, they present many differences, both at anatomical and chemical level. In fact, although they are all angiosperms, they have very different biological origins, belonging to significant different phylogenetic groups. Wheat is the classical example of an herbaceous crop (belonging to commelinids clade), eucalyptus is a typical hardwood and fast growing tree (belonging to the rosid clade), whereas olive tree is a slow-growing hardwood (belonging to the asterid clade). Their lignocellulose organization is significantly different, mainly with respect to hemicellulose and lignin composition. However, their hemicelluloses have the common trait of containing significant amounts of xylan.

As such, although it has already been proved that these materials can be successfully processed *per se* within the biorefinery framework (McIntosh et al., 2012; Negro et al., 2014; Pérez et al., 2008; Silva-Fernandes et al., 2015), their behavior in mixtures, specifically in the pretreatment/fractionation stage, is not known.

Hydrothermal pretreatments have been successfully applied at pilot demonstration and commercial scale for the biochemical upgrading of lignocellulosic materials (Larsen et al., 2008). These processes require no other reagents than water (Carvalho et al., 2008) and have a clear marked nature of a self-catalyzed process, from where it is derived the name of autohydrolysis. In fact, these processes selectively hydrolyze the hemicellulose fraction and hence are prominently influenced by hemicellulose composition (Garrote et al., 2002; Ruiz et al., 2013), in contrast to the acid catalyzed processes. The latter is considered less environmental-friendly by the use of catalyst and the higher neutralization agents when comparing with hydrothermal pretreatment, like autohydrolysis. In fact, autohydrolysis promotes lower liberation of compounds derived from lignin and lower cellulose and hemicellulose degradation, which favor bioconversion processes, due to the lower levels of microbial inhibitors generated during pretreatment (Garrote et al., 2001). The remaining solid fraction from autohydrolysis is enriched in cellulose and sulfur-free lignin. Cellulose can be subjected to enzymatic hydrolysis to produce glucose, while the lignin can be recovered, e.g. as phenolic extracts (after delignification) or directly used for energy (co-)generation (Larsen et al., 2008; Kang et al., 2013).

The main products found in the liquid fraction after autohydrolysis are oligosaccharides (OS), which cannot be directly metabolized by many microorganisms in bioconversion processes. Thus, a sequential hydrolysis process (post-hydrolysis step) can be applied to obtain a monosaccharides-stream to be used as sugar platform for the production of bio-based products. For this purpose, as compared to enzymatic post-hydrolysis, dilute-acid post-hydrolysis may have competitive advantages, like the lower costs of catalysts, the shorter reaction time required (Duarte et al., 2004, 2009; Garrote et al., 2001) and the higher yield, the later mainly because efficient hemicellulolytic cocktails are still under development to face hemicellulose heterogeneity among lignocellulosic residues (Mohanram et al., 2013). Although neutralization of post-hydrolysates is required, this procedure only deals with the liquid fraction obtained from autohydrolysis. Also, the combi-

nation of autohydrolysis and post-hydrolysis generates less sugar degradation products than a one-step acid-catalyzed pretreatment (Garrote et al., 2001).

In this work, three combinations of ER, WS and OP were subjected to autohydrolysis at different severities ($\log R_0$ from 1.73 up to 4.24) and its effect on the composition of liquid and solid fractions were evaluated. An empirical model was used as a tool to guide process optimization for each mixture and, at the higher OS content, a post-hydrolysis step of the liquid fraction was performed to evaluate maximal monosaccharides recovery.

2. Methods

2.1. Raw materials

ER are materials resulting from forest management practices, such as trimming and tree abatement, and were obtained from a commercial *Eucalyptus* farm (Mortágua, Portugal). They mainly consist of bark, branches and leaves and were provided already partially milled. WS was collected from an agricultural experimental station (Elvas, Portugal). OP, consisting of leaves and thin branches, were obtained from a local farmer (Ferreira do Alentejo, Portugal). Upon reception, ER, WS and OP were dried at 40 °C to obtain moisture content less than 10% (w/w). All feedstock were then individually grounded with a knife mill to particles smaller than 6.0 mm, homogenized and stored separately in plastic containers at room temperature.

These samples were used to prepare the different combinations of feedstock to be tested, as ER:WS:OP equal to 50:25:25, 25:50:25, or 25:25:50, also represented by 50ER, 50WS and 50OP, respectively. These combinations of feedstock were prepared, homogenized and then characterized as described below.

2.2. Autohydrolysis of different mixtures of eucalyptus residues, wheat straw and olive tree pruning

The autohydrolysis pretreatment was performed in a 0.6-L stainless steel reactor (Parr Instruments Company, Moline, Illinois, USA) equipped with a Parr PID temperature controller (model 4842). Each mixture (40 g dry basis) was loaded with water to obtain a liquid-to-solid ratio of 7 (g g^{-1}) and the agitation speed was set at 150 rpm. For each mixture, several non-isothermal conditions were tested, reaching final temperatures of 150, 170, 190, 195, 200, 205, 210, 215, 220 and 230 °C. After reaching the desired temperature, at an heating rate (above 100 °C) of 6.7, 6.2 and 6.8 °C/min, for 50ER, 50WS and 50OP, respectively, the reactor was rapidly cooled-down, by water circulation through a serpentine coil and its subsequent immersion in an ice bath, so that for the most severe condition cooling time was less than 2.5 min.

Liquid and solid fractions were separated using a hydraulic press (Sotel, Portugal) up to 200 kg/cm^2 and the liquid fraction recovered was filtered (Whatman No. 41 filter paper) to remove the remaining solids. The solid fraction was washed at room temperature with two volumes of water, filtered and dried at 40 °C before analysis of the chemical composition.

The effects of time and temperature on autohydrolysis were interpreted based on the severity factor, $\log R_0$ (Overend and Chornet, 1987):

$$R_0 = \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right) dt$$

where t is time (min), T the temperature (°C), and 14.75 an empirical parameter related with activation energy.

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