



Valorization of Eucalyptus wood by glycerol-organosolv pretreatment within the biorefinery concept: An integrated and intensified approach



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ABSTRACT

The efficient utilization of lignocellulosic biomass and the reduction of production cost are mandatory to attain a cost-effective lignocellulose-to-ethanol process. The selection of suitable pretreatment that allows an effective fractionation of biomass and the use of pretreated material at high-solid loadings on saccharification and fermentation (SSF) processes are considered promising strategies for that purpose. *Eucalyptus globulus* wood was fractionated by organosolv process at 200 °C for 69 min using 56% of glycerol-water. A 99% of cellulose remained in pretreated biomass and 65% of lignin was solubilized. Precipitated lignin was characterized for chemical composition and thermal behavior, showing similar features to commercial lignin. In order to produce lignocellulosic ethanol at high-gravity, a full factory design was carried to assess the liquid to solid ratio (3–9 g/g) and enzyme to solid ratio (8–16 FPU/g) on SSF of delignified Eucalyptus. High ethanol concentration (94 g/L) corresponding to 77% of conversion at 16FPU/g and LSR = 3 g/g using an industrial and thermotolerant *Saccharomyces cerevisiae* strain was successfully produced from pretreated biomass. Process integration of a suitable pretreatment, which allows for whole biomass valorization, with intensified saccharification-fermentation stages was shown to be feasible strategy for the co-production of high ethanol titers, oligosaccharides and lignin paving the way for cost-effective Eucalyptus biorefinery.

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1. Introduction

Lignocellulosic biomass conversion into biofuels (as bioethanol) is considered a promising alternative to replace fossil fuels, being one of investment priorities of European Union to attain a sustainable growth within Horizon 2020 [1,2]. In this sense, global demand of ethanol production could grow to exceed 125 billion liters [3]. Short-rotation plantations (as *Eucalyptus*) are forest lignocellulosic biomass considered one of the major renewable energy sources with a potential ethanol production of 7000 L/ha which could satisfy great part of the energetic needs [4]. In recent years, the research devoted to biomass bioconversion into bioethanol is gaining significant prominence, which is reflected in an increase of publishing in this field [5–7]. Although the investigation

in lignocellulosic ethanol has allowed the improvement of the technology the reduction of production costs is still mandatory for the industrial establishment of these processes. The intensification of the process can be the path to follow to attain an economic feasible process. The use of high solid loads and the integral use of all biomass fractions are key strategies for this purpose. Furthermore, for a proper evaluation of these strategies the integration of all stages of the process has to be considered.

For the integral use of all biomass fractions, the selection of a suitable pretreatment is determinant [8]. Organosolv pretreatment is considered a feasible process to enhance the enzymatic saccharification since it allows an effective high delignification and disruption of its recalcitrant structure. Moreover, organosolv processing is suitable for a biorefinery approach in which the obtained lignin has desired properties and the organic compound can be recovered easily [9]. Additionally, the use of glycerol (cheap industrial by-product from the biodiesel sector) on organosolv process has been lately suggested as a valuable green solvent, being an

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attractive approach for the treatment of biomass [10–14].

For further cost reduction, the intensification of the ethanol production process must be considered namely by using high-solid loads leading to high-gravity fermentations. In this context, the high-gravity fermentation, used in brewing and starch-based fermentation industries, produces a 10–15% (v/v) and improves the overall productivity as well as reduces capital cost and energy input comparing to normal gravity [15,16]. Therefore, the application of high-gravity technology in lignocellulosic ethanol plant could be an interesting strategy to achieve a cost-competitive process since the water economy of the process would be improved and lower costs of distillation would be involved. However, the implantation of this technology in the case of lignocellulosic feedstock implies the use of high biomass loadings in all process stages [17] which shows several operational limitations and challenges due to a lack of available water, the difficulty to mix and handle and the poor mass and heat transfer [18]. In consequence, the ethanol yields are usually low and the required enzyme loads are high [18]. In this context, only recently have few works reported efficient high concentration of cellulosic ethanol >4% (w/v) [19,20]. Moreover, high-gravity fermentation is related with stress responses in yeast, being essential for the use of industrial strains able to rapidly adjust their metabolism to harsh industrial conditions [16]. In this regard, industrial distillery environments as “cachaça” (Brazilian distilled beverage) are a good example of robust yeasts source for efficient high-gravity ethanol fermentation [21] and with higher thermotolerance, both features of great interest for the industry. The use of yeasts able to ferment at temperatures above 35 °C allows overcoming the main drawback of simultaneous saccharification and fermentation (SSF) process that is the difference between the optimal temperature of saccharification and fermentation [22]. SSF strategy implies the reduction of capital costs, reduces the enzyme loadings and increases the productivity [23–25]. Other alternative that could be considered is to carry out a pre-saccharification before the SSF process, also known as Pre-saccharification and Simultaneous Saccharification and Fermentation (PSSF) [26]. PSSF has been employed to reduce the viscosity of slurry at high solid loadings [27–29].

The strategy followed in this work shows a feasible process using glycerol as green solvent for the fractionation of *Eucalyptus globulus* wood (EGW) in order to obtain, within a biorefinery context, a pretreated biomass susceptible to be used as substrate at high-solid loadings (>30%) on saccharification and fermentation processes, as well as, a recovered lignin with similar features to commercial lignin. The effect of glycerol-organosolv pretreatment on pretreated EGW and organosolv lignin was evaluated by SEM, FT-IR, TGA and X-Ray. In addition, following a PSSF strategy, high-gravity ethanol production using a robust industrial and thermotolerant *Saccharomyces cerevisiae* strain was optimized under intensified conditions of low enzyme and high solid loadings by a full factorial design. Overall, the work carried out in this study opens new paths for cost-efficient lignocellulosic bioethanol production processes from a biorefinery approach by bridging and intensifying pretreatment, saccharification and fermentation stages.

2. Materials and methods

2.1. Raw material

Eucalyptus globulus wood (EGW) was collected from a paper mill (ENCE, Pontevedra, Spain), milled and stored in a dry place until to be used. The raw material was previously analyzed by Pereira et al. [30] following standard procedures for structural carbohydrates and lignin determination (NREL/TP-510-42618). The chemical

composition, expressed in g/100 g of raw material on dry basis, was: 44.70% of glucan; 16.01% of xylan; 1.09% of arabinan; 2.96% of acetyl groups; 27.70% of Klason lignin; 0.2% of ashes and 2.0% of extractives (see Table 1).

2.2. Pretreatment: glycerol-organosolv of EGW

Fig. 1 shows a scheme of the process followed in this work in which a biorefinery approach of EGW is proposed using organosolv process with glycerol-water as solvent in order to obtain in separated streams: cellulose for bioethanol production, hemicellulose as xylooligosaccharides and solubilized lignin. For that, the EGW was submitted to organosolv pretreatment in a 160 mL total volume batch cylinder stainless reactor and submerged in an oil bath with PID temperature controller, previously heated at 200 °C for 69 min for the pretreatment, the heating up and cooling were not taken into consideration. The EGW was mixed with 56% of glycerol-water at Liquid to Solid Ratio (LSR) = 10 g of glycerol-water/1 g of EGW on dry basis. The conditions of operation were chosen on basis of previous work [11]. After treatment, the delignified EGW was separated from liquid phase (black liquor) by vacuum filtration and washed with 10 g of NaOH (1%, w/w)/g of delignified EGW at 20 °C and two washes with approximately 1 L of distilled water/g of delignified EGW at 60 °C and 20 °C until pH = 7 (according to Dominguez et al. [14]) in order to remove adsorbed lignin from the pretreated solid (see Fig. 1). Washed delignified EGW was air-dried and quantified for solid yield (SY) determination (Table 1). The amount of liquid phase recovered (1046 g/100 g of EGW) was quantified considering the solubilized fraction of EGW (calculated as 100-SY). One aliquot of liquid phase (black liquor) was analyzed for derived-hemicellulose compounds (acetic acid, furfural and mono- and oligo-saccharides) concentration by acid post-hydrolysis treatment (121 °C, 4% w/w H₂SO₄ and 20 min) and quantified by HPLC. The solubilized lignin was precipitated adding 2 g of 0.3 M HCl/g of black liquor, recovered by centrifugation and kept overnight at 4 °C. The precipitated lignin (organosolv lignin) was dried at 50 °C to constant weight (15.6 g of lignin/100 g of raw material). In addition, acid soluble lignin in the liquor was measured (0.1%) by UV-vis spectroscopy (NREL/TP-510-42618).

2.3. Organosolv lignin characterization

2.3.1. Fourier-transform infrared (FT-IR)

The organosolv lignin and standard lignin (alkali lignin with low sulfonate content purchased in Sigma-Aldrich) were analyzed by a

Table 1
Solid Yield and composition data of EWG, Delignified EGW and liquid phase after organosolv pretreatment.

	EGW (g/100 g raw material, oven dry basis)	Delignified EGW (g/100 g of pretreated EGW, oven dry basis)
Solid Yield (SY)		54
a) Solid phase Composition		
Glucan	44.7	82.5
Hemicellulose		
Xylan	16.01	1.02
Arabinan	1.09	–
Acetyl groups	2.96	1.18
Klason Lignin	27.7	17.86
b) Liquid phase composition (g of monomer equivalent/L)		
Glucan		0.96
Xylooligosaccharides		11.08
Arabinooligosaccharides		0.09
Acetyl groups		4.91
Furfural		0.75

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