Bioresource Technology 167 (2014) 186–191

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhancement of enzymatic saccharification of *Eucalyptus globulus*: Steam explosion versus steam treatment

Raquel Martin-Sampedro *, Esteban Revilla, Juan C. Villar, Maria E. Eugenio

INIA-CIFOR, Forestry Products Department, Cellulose and Paper Laboratories, Ctra de la Coruña Km 7.5, Madrid 28040, Spain

HIGHLIGHTS

- Steam explosion and steam pre-treatments improved enzymatic saccharification.
- Higher hemicelluloses extraction was observed in steam explosion pre-treatments.
- Higher sugar yields were obtained after steam pre-treatments.
- The best results were obtained for samples with the lowest lignin content.

ARTICLE INFO

Article history:
Received 23 May 2014
Received in revised form 6 June 2014
Accepted 8 June 2014
Available online 16 June 2014

Keywords:
Autohydrolysis
Steam explosion
Enzymatic saccharification
Eucalyptus globulus

ABSTRACT

Steam explosion and steam pre-treatment have proved capable of enhancing enzymatic saccharification of lignocellulosic materials. However, until now, these methods had not been compared under the same operational conditions and using the same raw material.

Both pre-treatments lead to increased yields in the saccharification of *Eucalyptus globulus*; but results have been better with steam pre-treatments, despite the more accessible surface of exploded samples. The reason for this finding could be enzymatic inhibition: steam explosion causes a more extensive extraction of hemicelluloses and releases a greater amount of degradation products which can inhibit enzymatic action. Enzymatic inhibition is also dependent on the amount and chemical structure of lignin, which was also a contributing factor to the lower enzymatic yields obtained with the most severe pre-treatment. Thus, the highest yields (46.7% glucose and 73.4% xylose yields) were obtained after two cycle of steam treatment, of 5 and 3 min, at 183 °C.

1. Introduction

Lignocellulosic materials are a renewable resource of biomass that can be used as feedstocks in a biorefinery to be converted into a wide range of valuable products, including fuels, power, heat, chemicals and materials (Alvira et al., 2010; Carvalheiro et al., 2008). Second generation fuels, obtained from lignocellulosic materials, constitute an interesting alternative to bioethanol obtained from agricultural crops, since lignocellulosic materials do not compete with food crops and can be obtained from agroforestry residues. A variety of lignocellulosic materials have been suggested as suitable feedstocks, including *Eucalyptus globulus* wood (Castro et al., 2013; Gutsch et al., 2012; Romaní et al., 2013). *E. globulus* is a fast growing species widely used in Spain and Portugal to produce pulp, with high cellulose content, a limited lignin portion and hemicelluloses made up of acetylated glucuronoxylan.

One of the drawbacks of the utilization of lignocellulosic biomass in a biorefinery is the need of an efficient pre-treatment to eliminate physical and chemical impediments to subsequent steps, such as enzymatic hydrolysis. These pre-treatments are intended to improve formation of sugars, avoid degradation or loss of carbohydrates and formation of inhibitory products, and, last but not least, be cost-effective (Alvira et al., 2010). They can be classified as biological, physical, chemical and thermo-chemical pre-treatments, according to the different forces or energy consumed in the pre-treatment process. Among all of them, hydrothermal pre-treatments (such as hot water, steam or steam explosion pre-treatments) can be considered as green and competitive technologies to remove hemicelluloses from hardwood, since the sole presence of lignocellulosic feedstock and water in the reaction media prevents corrosion problems and formation of neutralization sludges (Garrote et al., 1999; Martín-Sampedro et al., 2011b). During these pre-treatments, the high-temperature steam/water releases acetic acids from the acetyl groups present in the hemicelluloses, which catalyze hydrolytic reactions in the

* Corresponding author. Tel.: +34 913476834; fax: +34 913476767.
E-mail address: martin.raquel@inia.es (R. Martin-Sampedro).

http://dx.doi.org/10.1016/j.biortech.2014.06.027
0960-8524/

wood polymers (Excoffier et al., 1991; Leschinsky et al., 2008; Li et al., 2005). Theses autohydrolysis reactions result in loss of hemicelluloses, which dissolve in hot water, and can be recovered in the liquid fraction to be further converted into value-added products.

Steam explosion and steam pre-treatment, as hydrothermal pre-treatments, lead to a similar autohydrolysis in the lignocellulosic material, being the main difference between these two treatments the rapid decompression that takes place at the end of steam explosion only. This decompression forces the fibrous material to rapidly expand, and the fibers and fiber bundles to come apart, generating a solid fraction with a more open structure (Ahvazi et al., 2007; Martín-Sampedro et al., 2011a,b) that may enhance the efficiency of subsequent treatments.

Although both pre-treatments have been discussed in different articles (Castro et al., 2013; Horn et al., 2011; Hu et al., 2013; Jedvert et al., 2012; Li et al., 2005; Romaní et al., 2010, 2013; Wu et al., 1999), until now, no direct comparison between them had been reported, except for a few assays by Li et al. (2005), which, nevertheless, did not apply the same severity factor in both methods. Therefore, the main objective of this study was to compare steam explosion and steam treatment at the same severity by evaluating their effects on the fractionation and subsequent enzymatic hydrolysis of *E. globulus*, and with the purpose of elucidating the separate effects of steam, on the one hand, (causing autohydrolysis in both pre-treatments), and of the "explosion", on the other hand. Furthermore, the influence of the severity factor in both pre-treatments was also studied.

2. Experimental

2.1. Raw material

E. globulus chips were kindly provided by La Montañanesa pulp mill (Torraspapel – Lecta Group, Spain). The size of the wood chips was approximately 2 × 3 × 0.5 cm. This material was air dried and then homogenized in a single stock (by conditioning inside polyethylene bags) to avoid differences in composition and water content. The chips were stored in polyethylene bags at 25 °C.

2.2. Steam explosion and steam treatments

Steam explosion and steam treatments were performed in a 26 L stainless steel digester capable of temperatures as high as 190 °C and a pressure of 1.37 MPa (14 kg-f cm^{-2}). This digester was connected to a blowing tank into which chips were discharged at the end of the treatment. It was also equipped with electrovalves for steam admission, and a ball valve of discharge. The steam generator was a Babcock Wanson VAP 250RR boiler, with a maximum steam production rate of 270 kg h^{-1} and a working pressure of 1.37 MPa.

In consistency with previous reports (Martín-Sampedro et al., 2011a, 2012), chips were first immersed in water at 25 °C for 16 h in order to improve the efficiency of the subsequent steam pre-treatments. In all of the experiments, 500 g of *E. globulus* chips were treated with steam at 183 °C (10 kg-f cm^{-2}). The variable operational conditions were: number of cycles of treatment (one or two), duration of the first cycle (5 or 10 min), and discharge pressure (6 kg-f cm^{-2} for steam explosion treatments or atmospheric pressure for steam treatments). When a second cycle was carried out, the pre-treated chips obtained in the first cycle were washed with cold water and then subjected to a second cycle of 3 min following the same procedure as in the first cycle. After treatment, the samples were thoroughly washed with water, dried at room temperature and ground in a Wiley mill to pass through a 0.2 mm screen.

The severity factor of each treatment was calculated according to the following equation (Eq. (1)), defined by Overend and Chornet (1987),

$$S_0 = \log\left(e^{\frac{T-100}{14.75}t}\right) \quad (1)$$

where T is the temperature (°C) and t the duration of the treatment (min).

2.3. Carbohydrate and lignin analysis

The compositions of the raw material and the solid fractions obtained in the pre-treatments were determined by standard analytical methods (National Renewable Energy Laboratory NREL/TP-510-42618). The extractives were determined as the soluble material after extensive Soxhlet extraction with ethanol. The extractive-free samples were subjected to quantitative acid hydrolysis in two steps to determine the carbohydrate composition. The sugar content was then analyzed in the hydrolysed liquid obtained using an Agilent Technologies 1260 HPLC fitted with a refractive index detector and an Agilent Hi-PlexPb column operated at 70 °C with Milli-Q water as mobile phase pumped at a rate of 0.6 mL min^{-1}. The solid residue remaining after the acid hydrolysis is considered acid insoluble lignin (Klason lignin). Additionally, the acid soluble lignin was quantified using UV spectrophotometry at 205 nm.

The compositions of the liquid fractions obtained from the pre-treatments were also determined. An aliquot (1 mL) was filtered through 0.45 μm nylon syringe membranes and used for direct HPLC determination of monosaccharides, acetic acid, furfural and hydroxymethylfurfural, using an Agilent Hi-PlexH column. A second aliquot of 25 mL was subjected to quantitative post hydrolysis with 4% H_2SO_4, at 120 °C, for 60 min, before HPLC analysis (according to NREL/TP-510-42623). Increments in the concentrations of monosaccharides and acetic acid caused by posthydrolysis were used to measure the concentrations of oligomers and acetyl groups bound to oligosaccharides, respectively.

2.4. Enzymatic hydrolysis of pre-treated wood

The solid fractions resulting from the different steam explosion and steam pre-treatments were subjected to enzymatic hydrolysis after grinding. All experiments were conducted in triplicate. A cellulolytic complex (Celluclast 1.5L), supplemented with β-glucosidase (Novozym 188), was added to a 5% sawdust suspension in 50 mmol L^{-1} sodium citrate buffer (pH 4.8). Both enzymatic mixtures were kindly provided by Novozymes (Bagsvaerd, Denmark). The enzyme doses were 15 FPU of Celluclast 1.5L and 15 IU of β-glucosidase per gram of dry sample. Enzymatic hydrolysis was performed in a thermostatic rotary shaker at 50 °C and 120 r.p.m. for up to 72 h. Samples of 1.5 mL were taken after 6, 24, 48 and 72 h of incubation to evaluate the release of sugar by High Pressure Liquid Chromatography (HPLC). These liquid samples were heated in boiling water for 10 min and, after cooling, filtered throw a 0.45 μm nylon syringe filter. Then, samples were used for direct determination of monosaccharides, using an Agilent Technologies 1260 HPLC fitted with a refractive index detector and an Agilent Hi-PlexH column operated at 65 °C with a mobile phase containing 5 mmol L^{-1} of sulfuric acid pumped at a rate of 0.6 mL min^{-1}.

3. Results and discussion

3.1. Steam explosion and steam pre-treatments

The chemical composition of the solid obtained after each pre-treatment is showed in Table 1. This table also lists the solid yield,