Bioresource Technology 162 (2014) 175–183

Contents lists available at ScienceDirect

Bioresource Technology

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

Steam explosion of oilseed rape straw: Establishing key determinants of saccharification efficiency

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HIGHLIGHTS

- Pretreatment of OSR straw affects cellulase adsorption and hydrolysis in several ways.
- Pectin reduces cellulase binding and initial hydrolysis rates.
- Lignin increases initial hydrolysis duration probably by maintaining open structure.
- Maximum hydrolysis yield requires xylan removal.
- Milling enhances saccharification efficiency at low severity pretreatments.

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 19 March 2014
Accepted 21 March 2014
Available online 31 March 2014

Keywords:
Brassica napus
Cellulase binding
Pretreatment
Rapeseed straw
Enzymatic hydrolysis

ABSTRACT

Oilseed rape straw was steam exploded into hot water at a range of severities. The residues were fractionated into solid and liquid phases and chemically characterised. The effect of steam explosion on enzymatic hydrolysis of the water-insoluble fractions was investigated by studying initial cellulase binding and hydrolysis yields for different cellulase doses. Time-course data was modelled to establish rate-dependent differences in saccharification as a function of pretreatment severity and associated chemical composition. The study concluded: (1) the initial hydrolysis rate was limited by the amount of (pectic) uronic acid remaining in the substrate; (2) the proportion of rapidly hydrolysable carbohydrate was most closely and positively related to lignin abundance and (3) the final sugar yield most closely related to xylan removal from the substrate. Comparisons between milled and un-milled steam exploded straw highlighted the influence that physical structure has on hydrolysis rates and yields, particularly at low severities.

1. Introduction

Releasing fermentable sugars from lignocellulose would allow high-value products to be generated from abundant, cheap and renewable biomass sources (Waldron, 2010). Extracting monomeric sugars from biomass currently requires relatively expensive processing regimes of sequential pretreatment and enzymatic hydrolysis before fermentation to the desired product (Kumar and Murthy, 2011). Establishing economical pretreatment and saccharification of biomass is therefore an essential prerequisite for commercial exploitation.

Some of the most promising biomass sources are those derived from crop waste streams as they are abundant, low value and do not compete directly with food crops. Oilseed rape (OSR) produces a particularly attractive agricultural residue as the crop is primarily cultivated for seed oil. However, a considerable amount of above-ground biomass produced by the plant remains in the straw (≈70%) which is poorly exploited. This residual straw contains relatively high glucose concentrations (27–37% DWB) and therefore regarded as a good potential lignocellulosic feedstock (Castro et al., 2011; Diaz et al., 2010; Jeong and Oh, 2011; Lu et al., 2009, 2011; Mathew et al., 2011a,b; Petersson et al., 2007; Wi et al., 2011). Many pretreatment techniques have been explored using OSR straw, including microwave (Lu et al., 2011), wet oxidation (Petersson et al., 2007), hydrothermal (Diaz et al., 2010; López-Linares et al., 2013), dilute acid (Castro et al., 2011; Lu et al., 2009; Mathew et al., 2011a), alkali (Mathew et al., 2011b), and autocatalytic 'popping' (Wi et al., 2011).

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http://dx.doi.org/10.1016/j.biortech.2014.03.115
0960-8524/

Steam explosion has many potential advantages over other pretreatment methodologies including: lower capital costs, higher water efficiencies, high substrate loadings, better overall energy use and consequently, a lower potential product price (Conde-Mejía et al., 2012; Kumar and Murthy, 2011). Recently, Ryden et al. (2014) demonstrated the predictive effect of steam explosion (without substrate washing) on the main polymer groups and saccharification of OSR straw after 22 h hydrolysis using a commercial cellulase (Ryden et al., 2014).

Pretreatment not only increases the overall saccharification yield, but also the efficiency of the cellulase in producing monomeric sugars (Arantes and Saddler, 2011). Although pretreatment is well known to have gross effects on biomass composition, the exact mechanisms by which hydrolysis is improved are not well understood. Lignocellulose is a heterogeneous composite made from carbohydrate and phenolic components and can be hydrolysed using multi-component cellulase cocktails. As a result, biomass hydrolysis does not follow typical enzyme kinetics. Instead, the final glucose yield released from a substrate is determined by a number of hydrolysis stages, limited by various interactions between multiple cellulases and the solid substrate (Bansal et al., 2009).

The aim of this study has been to understand the relationships between pretreatment severity, changes in substrate chemical composition, and the rate, extent and efficiency of cellulase binding and enzymatic hydrolysis. Water insoluble pretreated OSR straw was assessed for key differences in initial cellulase binding and hydrolysis yields produced for varying cellulase doses. Rate-dependent disparities in hydrolysis were investigated by comparing the hydrolysis yields achieved at various points during saccharification. These parameters were then compared to the composition of the pretreated materials to gain a greater nsight into the chemical determinants of saccharification performance.

2. Methods

2.1. Materials

OSR straw (stems and empty pods) was sourced from Hemp Technology Ltd. Suffolk, UK (52°21′15.7″N 1°30′35.7″E). Straw was harvested in 2011, chipped into <350 mm pieces, dust extracted and baled. The straw was stored as a 20 kg bale in a dry, unheated room before analysis (<1 year). No further size-reduction was conducted before steam explosion.

The cellulase cocktail used in this study was Accelerase® 1500 (Genencor, UK) with a stock solution cellulase activity of 72.9 FPU/ml – measured following Ghose (1987) and a protein concentration of 12.92 g/L determined using the bicinchoninic acid (BCA) assay. Unless otherwise stated, all chemicals used were analytical grade, purchased from Sigma–Aldrich, UK.

2.2. Steam explosion of oilseed rape straw

Samples of OSR straw (1 kg FW) were steam exploded into hot water (6.6 L) at a range of pretreatment severities (9.01–26.94 bar, 180–230 °C, 10 min) using a Cambi™ Steam Explosion Pilot Plant (Cambi, Asker, Norway). A sample of chipped straw (1 kg) was heated with pressurised steam in a sealed 30 L vessel, and held at the desired temperature for 10 min. After this time, the contents of the heating chamber were rapidly evacuated into a cyclone (separating biomass from steam) and solid components deposited in a hopper containing 6.6 L of water. The heating chamber was then cleared twice by applying 2–3 bar of pressure before removing the pretreated material.

The pretreated biomass was then collected and immediately filtered through a 100 μm nylon mesh bag in a low speed centrifuge. The liquor and insoluble pretreated solid were collected in their entirety and frozen to prevent microbial growth (−40 °C). The steam explosion unit was extensively flushed between runs to prevent any carry-over of material to subsequent pretreatments.

2.3. Chemical composition of the untreated and pretreated solids

The dry matter content of the pretreated biomass was established using an infrared drying balance (Mettler LP16, Mettler–Toledo, Belgium) drying duplicate samples (0.5 g) at 105 °C, to constant mass. Ash content was calculated gravimetrically, after charring samples in a muffle furnace (120 °C, 2 h → 250 °C, 4 h → 500 °C, 24 h; ramping at 5, 2 and 5 °C/min respectively).

A sample of each steam exploded solid was freeze-dried and subjected to the following analyses. Klason lignin content was calculated gravimetrically after acid hydrolysis (residue after 72% H_2SO_4, 20 °C, 3 h, followed by dilution to 1 M, 100 °C, 2.5 h, and filtered through a No. 4 sintered glass filter) and the abundance of acid-insoluble ash subtracted from the total. The sugar composition of the freeze-dried solids was established by converting the acid-hydrolysed-sugars to their aditol acetate derivatives and quantifying their abundance by gas chromatography (Blakeney et al., 1983). 2-deoxyglucose was used as an internal standard. Uronic acid content was established colorimetrically after a milder hydrolysis regime (72% H_2SO_4, 20 °C, 3 h → dilution to 1 M, 100 °C, 1 h) following (Blumenkrantz and Asboe-Hansen, 1973).

2.4. Analysis of liquid fractions

The pH of the steam exploded liquors was determined directly after collection, using a digital pH meter (Jenway 3020, Jenway Ltd., UK). The liquor was then syringe filtered (0.22 μm, Millex®, Millipore, USA) before quantifying the abundance of monomeric sugar and degradation products by HPLC using a Flexar® FX-10 UHPLC instrument (Perkin Elmer, UK) equipped with a refractive index detector and Aminex HPX-87P carbohydrate analysis column (Bio-Rad Laboratories Ltd, UK) (85 °C, mobile phase Milli-Q water, flow rate 0.6 ml/min). Organic acid concentrations were established using the same HPLC system fitted with an Aminex HPX-87H organic acid analysis column (Bio-Rad Laboratories Ltd., UK) (65 °C, mobile phase 5 mM H_2SO_4, flow rate 0.5 ml/min).

2.5. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were collected in the 800–4000 cm^{-1} region for both the insoluble pretreated solid and liquors using a dynamic alignment attenuated total reflectance FT-IR spectrophotometer (Bio-Rad FTS 175C, Bio-Rad Laboratories, Cambridge, USA), equipped with a Golden Gate™ ATR accessory (speed 10 kHz, filter 5, UDR 2, resolution 2 cm^{-1}, sensitivity 1, 64 scans). The solid fraction was analysed using air as the reference medium and liquid fractions were analysed against ultrapure water (MilliQ). Values within the fingerprint region (800–1800 cm^{-1}) were extracted, baseline anchored to 1800 cm^{-1} and areas normalised. Triplicate spectra were taken for each sample and averaged.

2.6. Biomass preparation before saccharification assessment

The water-insoluble steam exploded biomass was homogenised by cryogenic milling (maximum impact frequency for 3 min, SPEX 6700 freezer/mill, Spex Industries, NJ) so consistent suspension of the solids could be achieved. All substrates, irrespective of pretreatment severity were reduced to sub-cellular fractions of similar size (mean = 41–73 nm, median = 39–85 nm) after