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Hydrothermal pre-treatment of rapeseed straw

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ABSTRACT

As a first step for ethanol production from alternative raw materials, rapeseed straw was studied for fermentable sugar production. Liquid hot water was used as a pre-treatment method and the influence of the main pre-treatment variables was assessed. Experimental design and response surface methodology were applied using pre-treatment temperature and process time as factors. The pretreated solids were further submitted to enzymatic hydrolysis and the corresponding yields were used as pre-treatment performance evaluation. Liquid fractions obtained from pre-treatment were also characterized in terms of sugars and no-sugar composition. A mathematical model describing pre-treatment effects is proposed. Results show that enzymatic hydrolysis yields near to 100% based on pretreated materials can be achieved at 210–220 °C for 30–50 min, equivalent to near 70% of glucose present in the raw material. According to the mathematical model, a softer pre-treatment at 193 °C for 27 min results in 65% of glucose and 39% of xylose available for fermentation.

1. Introduction

The growing interest on renewable energy sources has made attention to be devoted especially on raw materials that present no competence with food or feed applications, like a number of agricultural residues showing to date no alternative uses. Furthermore, the need for disposal of such materials makes the study of a new destiny for them even more attractive.

The use of agricultural residues as raw material for ethanol and other chemicals production has been reported, e.g., olive tree biomass (Cara et al., 2008a), wheat straw (Pérez et al., 2008), sunflower stalks (Ruiz et al., 2008), cotton stalks (Shi et al., 2009), rice straw (Karimi et al., 2006), corn stover (Wyman et al., 2005), and many others (Kadar et al., 2007). Pre-treatment is a key step for using agricultural residues in these processes because of the recalcitrance of cellulose and the physico-chemical barrier formed by both lignin and hemicelluloses. To improve the accessibility of enzymes to cellulose different pre-treatment methods have been proposed (Sun and Cheng, 2002). Hydrothermal methods benefit from the use of no chemical agents other than water, thus reducing expensive recovery costs and being more environmentally friendly technologies (Hashaikeh et al., 2007; Garrote et al., 1999). As a result of the pre-treatment, the solid residue is enriched in cellulose, which remains almost unaltered during the process (Garrote et al., 2008) enabling its use with several objectives, e.g., ethanol production. Liquid hot water (LHW) treatment, a hydrothermal pre-treatment method usually applied to agricultural residues (Cara et al., 2007), results in higher hemicellulose sugar recovery and lower fermentation inhibiting hydrolysates than steam explosion pre-treatment (Allen et al., 2001; Laser et al., 2002). Typical conditions for LHW pre-treatment include temperatures around 200 °C for a few minutes.

Rapeseed (*Brassica napus*) has traditionally been grown for the production of animal feed and vegetable oil for human consumption. In the last years, an increasing fraction of rapeseed oil has been used as raw material for biodiesel production. According to FAO (FAO, 2008), over 30 million hectares of rapeseed were cultivated worldwide in 2007. After seed harvesting, rapeseed straw, left behind on the fields, must be eliminated.

There are relatively little reports on the use of rapeseed residues as renewable energy source. Some reports deals with thermal methods; for example, Karaosmanoglu et al. (1999) used pyrolysis of the straw and stalk of the rapeseed plant for biofuel production. Zabaniotou et al. (2008) reported on the integrated utilization of rapeseed suitable to Greek conditions for biodiesel production and parallel use of its solid residues for energy and second generation biofuels production via fast pyrolysis. Reports dealing with rapeseed straw as raw material for ethanol production are also rare. The use of sulphuric acid-catalyzed pre-treatment with rapeseed straw has been reported (Lu et al., 2009). Li et al. (2009) reported on rapeseed stover pre-treatment with phosphoric acid–acetone for ethanol production by means of simultaneous saccharification and fermentation. Biogas or ethanol production has also been reported (Petersson et al., 2007).

The objective of this work is to assess the possibilities of using rapeseed straw as a source of fermentable sugars by means of liquid hot water pre-treatment. The main operation variables, e.g., pre-treatment temperature and process time, are evaluated using an experimental design approach. The enzymatic hydrolysis

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yields of the pretreated material as well as the sugar yield in the liquid fractions issued from pre-treatment are used as pre-treatment performance evaluation.

2. Methods

2.1. Raw material

Rapeseed straw was locally collected after seed harvest. Then, the raw material was air-dried at room temperature to equilibrium moisture content of about 10%, milled using a laboratory hammer mill (Retsch) to a particle size smaller than 1 cm, homogenised in a single lot and stored until used.

2.2. LHW pre-treatment

Liquid hot water pre-treatment was performed in a laboratory scale stirred Parr reactor. The reactor has a total volume of 1 L, with an electric heater and mechanic agitation. The temperature/speed controller is a combination of furnace power control and motor speed control with tachometer. Cooling water was circulated through a serpentine coil to cool the reactor content at the end of each run. Thirty six grams of rapeseed straw (dry basis) and 600 mL water were used for each pre-treatment trial. Both water and raw material were initially at room temperature. Agitation was set at 350 rpm. The average heating rate was 5 °C/min. Pre-treatment time was initiated when the selected pre-treatment temperature was reached. After treatment, the reactor was removed from the heating jacket and cooling water was charged through the serpentine coil. The content of the reactor cooled down to 80 °C in approx 5 min. The reactor was kept sealed, and the slurry agitated until the reactor was cooled to about 40 °C. Then the wet material was filtered for solid and liquid recovery.

The water-insoluble solids were washed out with water and analyzed for hemicellulosic sugars, glucose and acid-insoluble lignin content, and used as substrate in enzymatic hydrolysis tests. Liquid fraction issued from pre-treatment (hydrolysate) was analyzed for sugars, acetic acid and sugar-degradation products.

2.3. Experimental design

Rapeseed straw was LHW-pretreated at 13 different operational conditions according to a rotatable central composite design ($\alpha = 1.414$), as shown in Table 1. In addition, run 14 was performed to verify results derived from the model (see Section 3). Center values and intervals for both pre-treatment temperature and time were chosen based on previous experience with agricultural residues to ensure a broad range of responses. Pre-treatment experiments were performed in random order. The experimental data were analyzed by the statistical software Design Expert 7.0, Stat-Ease Inc., Minneapolis, USA.

Table 1
Experimental design for LHW pre-treatment of rapeseed straw.

Run number	Temperature (°C)		Time (min)	
	Coded value	Real value	Coded value	Real value
1	−1	170	−1	10
2	+1	210	−1	10
3	0	190	0	30
4	0	190	0	30
5	−1	170	+1	50
6	0	190	0	30
7	+1	210	+1	50
8	+1.414	218.3	0	30
9	0	190	0	30
10	0	190	−1.414	1.7
11	0	190	+1.414	58.3
12	0	190	0	30
13	−1.414	161.7	0	30
14	+2	230	−1	10

2.4. Enzymatic hydrolysis tests

The washed water-insoluble residue of pretreated rapeseed straw was enzymatically hydrolysed by a cellulolytic complex (Celluclast 1.5 L) kindly provided by Novozymes A/S (Denmark). Cellulase enzyme loading was 15 Filter Paper Units (FPU)/g substrate. Fungal β-glucosidase (Novozym 188, Novozymes A/S) was used to supplement the β-glucosidase activity with an enzyme loading of 15 International Unit (IU)/g substrate. Enzymatic hydrolysis was performed in 0.05 M sodium citrate buffer (pH 4.8) at 50 °C on a rotary shaker (Certomat-R, B-Braun, Germany) at 150 rpm for 72 h and at 5% (w/v) pretreated material concentration. Samples were taken every 24 h for glucose concentration determination. All enzymatic hydrolysis experiments were performed in duplicate (standard deviations were in all cases <3%) and average results are given.

2.5. Analytical methods

The composition of raw material was determined according to the National Renewable Energy Laboratory analytical methods for biomass (NREL, 1994, 1996, 1998). Prior to other determinations, raw material was extracted consecutively with water and with ethanol (two-step extraction procedure). After the first step, the sugar composition of the water-extract was determined by high performance liquid chromatography (HPLC) in a Varian Prostar liquid chromatograph with refractive index detector. A Transgenomic CHO-682 carbohydrate analysis column operating at 80 °C with ultrapure water as a mobile-phase (0.4 mL/min) was used. Free and oligomeric sugar composition was determined before and after a posthydrolysis process consisting in a treatment with sulfuric acid (3% v/v) at 121 °C and 30 min. The cellulose and hemicellulose content of the extracted solid residue was determined based on monomer content measured after a two-step acid hydrolysis procedure to fractionate the fiber. A first step with 72% (w/w) H_2SO_4 at 30 °C for 60 min was used. In a second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121 °C for 1 h. This hydrolysis liquid was then analyzed for sugar content by HPLC as described above. The remaining acid-insoluble residue is considered as acid-insoluble lignin (AIL).

Following LHW-pre-treatment, the composition of solid fraction was determined as described for raw material except that no extraction is used. The sugar content (glucose, xylose, arabinose, mannose and galactose) of the liquid fraction after pre-treatment (prehydrolyzate) was determined by HPLC using the system described above. The inhibitor composition (acetic acid, formic acid, furfural and HMF) was determined using the HPLC system with refractive index detector mentioned above; a Bio-Rad HPX-87H column at 65 °C temperature was used. The mobile phase was 5 mM H_2SO_4, at a flow rate of 0.5 mL/min. Glucose concentration from enzymatic hydrolysis samples was measured by HPLC with the above described Varian equipment. All analytical determinations were performed in duplicate and average results are shown. Relative standard deviations were in all cases below 5%.

3. Results and discussion

3.1. Raw material composition

The composition of the rapeseed straw is summarized in Table 2 and agrees well with that reported by other authors (Lu et al.,