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Optimization of uncatalyzed steam explosion pretreatment of rapeseed straw for biofuel production

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HIGHLIGHTS

- Ethanol production from steam exploded rape straw at high solids loading is reported.
- Operation at high solids loading (20% w/v) allows complete conversion of cellulose.
- High ethanol concentrations (5% v/v) are obtained, allowing industrial operation.

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ABSTRACT

Rapeseed straw constitutes an agricultural residue with great potential as feedstock for ethanol production. In this work, uncatalyzed steam explosion was carried out as a pretreatment to increase the enzymatic digestibility of rapeseed straw. Experimental statistical design and response surface methodology were used to evaluate the influence of the temperature (185–215 °C) and the process time (2.5–7.5 min). According to the rotatable central composite design applied, 215 °C and 7.5 min were confirmed to be the optimal conditions, considering the maximization of enzymatic hydrolysis yield as optimization criterion. These conditions led to a maximum yield of 72.3%, equivalent to 81% of potential glucose in pretreated solid. Different configurations for bioethanol production from steam exploded rapeseed straw were investigated using the pretreated solid obtained under optimal conditions as a substrate.

As a relevant result, concentrations of ethanol as high as 43.6 g/L (5.5% by volume) were obtained as a consequence of using 20% (w/v) solid loading, equivalent to 12.4 g ethanol/100 g biomass.

1. Introduction

Biofuels production can be a sustainable alternative to fossil energy sources as well as a solution to combat climate change (Gupta, 2014). Lignocellulose biomass like forest and agricultural residues can be considered an interesting feedstock for second generation bioethanol production without competition with food or feed uses and more environmentally friendly. They are renewable, abundant, cheap and they can be alternative renewable resources. As a source of raw materials, rapeseed opens up a multitude of possibilities in the food and feed industry, as well as in the energy and material sectors (UFOP, 2014). The land surface planted with rapeseed in the world has increased from approximately 26 million hectares in 2000 to more than 36 million hectares in 2013 (FAOSTAT, 2015). Rapeseed straw can be left on the fields to maintain soil health. Currently, the greatest demand for straw is for burning in power stations (Ryden et al., 2014). Besides, this cellulosic biomass may constitute a renewable energy resource for fuel ethanol production by biological conversion.

The first step in the conversion of lignocellulosic biomass into fuels or chemicals typically involves a biomass pretreatment step in order to make the cellulose easier to hydrolyze by removing the hemicellulose, reducing the crystallinity of cellulose, and increasing the surface area (Wettstein et al., 2012). Effective pretreatment is fundamental to a successful enzymatic hydrolysis (EH) since thus the saccharification of the pretreated feedstock is enhanced by improving the accessibility of enzymes to the cellulose fibers (Limayem and Ricke, 2012). Steam explosion is recognized as a low-cost option for agricultural residues pretreatment with significantly lower environmental impact since the addition of external chemicals is not necessary (Singh et al., 2015). Moreover, it is considered as an effective pretreatment that causes the breakdown of lignocellulose structure which results in the

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partial hydrolysis of hemicellulose and disruption of the lignocellulosic matrix by the sudden pressure drop (Romaní et al., 2013). Although an acid presoaking can improve the treatment, the highly acetylated nature of some lignocellulosic materials allows for uncatalyzed (or autocatalyzed) steam pretreatment due to the release of acetyl groups and formation of acetic acid (Alvira et al., 2010). In addition, steam explosion pretreatment without external catalyst addition avoid the additional chemical costs and associated problems derived of equipment corrosion. Thus, the uncatalyzed alternative means a significantly lower environmental impact for the chemical utilization (Romaní et al., 2013). Uncatalyzed steam explosion has previously been shown to be very effective for the pretreatment of others lignocellulosic materials like wheat straw (Ballesteros et al., 2006), *Eucalyptus globulus* (Romaní et al., 2013) or corn stover (Lu et al., 2010).

The performance of the subsequent enzymatic hydrolysis and fermentation at a high solids loading allows to increase the final ethanol concentration. It is essential to improve the economy of the process by reducing the energy consumption in the distillation step (Larsen et al., 2008). However, mixing difficulties in the reactor due to the high viscosity can occur (Sassner et al., 2006) and the yeast can be inhibited reducing ethanol yield in the simultaneous saccharification and fermentation step or even the cessation of ethanol production (Hoyer et al., 2013).

This works deals with the steam explosion pretreatment of rapeseed straw, evaluating the effect of the temperature and process time by the response surface methodology. The performance of enzymatic hydrolysis and fermentation processes at high solids loading was tested using the solid pretreated obtained under optimal steam explosion conditions as a substrate.

2. Methods

2.1. Raw material

Rapeseed straw (8% moisture content) was collected in Seville, Spain, after seed harvest, air-dried at room temperature, milled using a laboratory hammer mill (Retsch, SM-100, Haan, Germany) to a particle size smaller than 1 cm and stored in a dry place until use.

2.2. Steam explosion pretreatment

Rapeseed straw was pretreated by steam explosion, without previous impregnation, in a pilot unit based on Masonite technology and equipped with a 2-L reaction vessel, as described elsewhere (Ballesteros et al., 2006). The reactor was charged with 300 g (dry matter) of feedstock per batch and heated to the desired temperature, directly with saturated steam. The selected temperature was reached in 40–50 s and then, time counting was initiated. After the explosion, the material was recovered in a cyclone, cooling to about 40 °C and then filtered for liquid and solid recovery. The solid fractions (water-insoluble solids, WIS) were washed with 5 L distilled water to remove the remaining prehydrolysate and stored wet in plastic bags. The WIS were analyzed for hemicellulosic sugars, glucose and lignin content and used as substrates in enzymatic digestibility tests. Liquid fractions (prehydrolysates) were analyzed for sugars, acetic acid, phenolic compounds and sugar degradation products as formic acid, furfural and 5-hydroxymethylfurfural (HMF).

2.3. Statistical design of experiments

Rapeseed straw was pretreated at different operational conditions according to a rotatable central composite design ($\alpha = 1.414$) including one point and four replicates at the center of domain selected for each factor under study (13 runs). Pretreatment assays were performed in random order. Temperature (in the range 185–215 °C) and time (2.5–7.5 min) were selected as variables. Center values and intervals were selected based on previous experience. The coded and uncoded values of factors in the rotatable central composite design are shown in Table 1. The experimental data were analyzed via response surface methodology by the statistical software Design-Expert 8.0.7.1, Stat-Ease Inc., Minneapolis, USA.

2.4. Enzymatic hydrolysis tests

The washed WIS fractions of rapeseed straw obtained after pretreatment were used as substrates for enzymatic hydrolysis assays. These WIS fractions were hydrolyzed with a cellulolytic complex (Celluclast 1.5 L) kindly provided by Novozymes A/S (Denmark), which contains an activity of 69 filter paper units (FPU)/mL. The cellulase enzyme loading was 15 FPU/g substrate. To supplement β-glucosidase activity of cellulases, fungal β-glucosidase (Novozym 188, Novozymes A/S), with an activity of 530 international units (IU)/mL was added, at an enzyme loading of 15 IU/g substrate. The pH was adjusted to 4.8 with 0.05 M sodium citrate buffer and enzymes were added to the pretreated substrate (5% w/v dry basis) for a total working volume of 20 mL in 100 mL Erlenmeyer flasks. Triplicate reaction flasks were incubated at 50 °C in an orbital shaker (Certomat-R, B-Braun, Germany) at 150 rpm for 72 h. Two milliliter samples were withdrawn at 24, 48 and 72 h, and they were centrifuged at 10,000*g* (Sigma 1–14 Centrifuge) for 10 min. Glucose and xylose concentrations in the sample supernatant were determined by HPLC.

Once the steam explosion conditions were optimized to maximize the enzymatic hydrolysis yield, different substrate and enzyme loadings were tested. Then, the WIS fraction obtained under optimal conditions was washed and submitted to enzymatic hydrolysis tests as described above.

In order to evaluate the performance of enzymatic hydrolysis tests, two parameters were determinate as follows. Saccharification efficiency (SE) was calculated as the ratio of grams of glucose released by enzymatic hydrolysis per 100 g glucose in the WIS, and the enzymatic hydrolysis yield (Y_{EH}) was referred to the glucose content in the raw material.

All enzymatic hydrolysis experiments were performed in triplicate. Average results and standard deviations are given.

2.5. Microorganism, medium and yeast cultivation

Saccharomyces cerevisiae (Fermentis Ethanol Red, Cedex, France) was used for fermentation assays. Yeast was maintained on solid

Table 1
Operational conditions assayed expressed as dimensional and dimensionless independent variables.

Run	Temperature (°C)		Time (min)	
	Real value	Coded value	Real value	Coded value
1	221.21	+1.414	5.00	0
2	200.00	0	8.54	+1.414
3	185.00	−1	2.50	−1
4	200.00	0	5.00	0
5	215.00	1	7.50	1
6	200.00	0	5.00	0
7	185.00	−1	7.50	1
8	215.00	1	2.50	−1
9	200.00	0	5.00	0
10	200.00	0	5.00	0
11	200.00	0	5.00	0
12	200.00	0	1.46	−1.414
13	178.79	−1.414	5.00	0