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Bioethanol production from rapeseed straw at high solids loading with different process configurations

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HIGHLIGHTS

- Rapeseed straw is attracting great interest as raw material for fuel production.
- Three strategies are compared for the first time in rapeseed straw.
- Operation at high solids loading (20%) allows feedstock full use.
- Ethanol concentrations as high as 5% are obtained.

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ABSTRACT

Rapeseed is an important source of oil for biodiesel production. Nevertheless, the residues of the cultivation are lacking of practical applications. As a lignocellulosic material, their conversion into ethanol can be of interest. In this work, different process configurations, separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and prehydrolysis and simultaneous saccharification and fermentation (PSSF), were compared at high solids loading (7.5%, 15% and 20% w/v) to produce ethanol from rapeseed straw pretreated by sulfuric acid.

Results show that the highest ethanol concentration (39.9 g/L) was obtained from SHF configuration at the highest substrate loading (20% w/v). This product concentration is high enough for distillation purposes from an economic point of view. The final ethanol concentrations and yields did not differ significantly between SSF and PSSF regardless of the solids loading and, for 7.5% and 15% (w/v) solids loading were slightly higher than those attained in the SHF. However, at the highest solids loading the separate process appears to be more favorable.

1. Introduction

Over 34 million hectares of rapeseed were cultivated word wide in 2012 [1], mainly dedicated to oil production for the biodiesel industry. Rapeseed straw is a lignocellulosic agricultural residue with relatively high sugar content – near 60% – that makes it an interesting raw material for second-generation ethanol production [2]. Ethanol from rapeseed straw would contribute to the biorefinery development based in this crop [3], whose concept involved the exploitation of the largest possible part of the biomass [4].

In recent years, numerous research efforts have been made in the technologies for improving the production of ethanol from different lignocellulosic materials, being bioprocesses based on enzymatic hydrolysis the most interesting alternative [5,6]. Pretreatment, hydrolysis and fermentation are the main steps involved in these processes. Increasing solids loading in both the hydrolysis and fermentation steps is one of the most important challenges to make biofuels production more economical [4] because of the reduction in ethanol distillation cost [7]. However, increasing solids concentration has also associated drawbacks as larger levels of inhibiting compounds [8], end-product inhibition [9], diffusional enzyme problems [10], stirring and mixing limitations by viscosity increase [11] or possible mass transfer limitations appearing above 20% insoluble solids concentration [12].

The production of ethanol can be accomplished following several process strategies, and it is useful to compare them in order to select the best one in this particular case. They include separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and prehydrolysis and simultaneous saccharification and fermentation (PSSF). SHF involves two sequential steps, enzymatic saccharification of pretreated cellulose and sugars fermentation to ethanol. This configuration allows working at optimal operating conditions for enzymes and microorganisms. SSF

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process consists in performing hydrolysis and fermentation in a single step, which offers the advantage of preventing the end-product inhibition by enzymatic saccharification. In addition, the use of a single reactor eliminates the need for solid–liquid separation devices simplifying the industrial implementation of the process [13]. However, an important drawback for SSF is the difficulty to perform yeast recirculation because the final slurry contains also other solids mainly lignin [7]. A major SSF drawback is the mismatch between the optimum temperature of enzymes and microorganism [14]. PSSF configuration could be conducted in an attempt to partially solve this disadvantage. This consists of a brief enzymatic hydrolysis carried out at the optimum temperature followed by an SSF process conducted at a lower temperature to facilitate sugar conversion to ethanol. This strategy also aims to reduce viscosity of the solid–liquid mixture prior to the addition of the microorganism [15].

Rapeseed straw was investigated as feedstock for ethanol production with promising results. Different pretreatment strategies were assayed with this raw material, namely liquid hot water [16], acid prehydrolysis with H_2SO_4 [17,18], H_3PO_4 [19] or H_3PO_4–acetone [20], wet oxidation [21] or H_2O_2 pretreatment [3,22]. However, relatively low solids loading for enzymatic saccharification was employed in most cases. The aim of this work is to compare different strategies for the production of ethanol from pretreated rapeseed straw. SHF, SSF, PSSF configurations were evaluated, including fed-batch substrate and enzyme feedings. A range of solids loading from 7.5% to 20% (w/v) was investigated with the objective of achieving high concentrations of ethanol which plays a major role regarding a potential industrial scale process implementation.

2. Materials and methods

2.1. Raw material and sulfuric acid pretreatment

Rapeseed straw was collected in the province of Granada (south of Spain) after seed harvest. Prior to sulfuric acid pretreatment, rapeseed straw was milled, using a laboratory hammer mill (Retsch, Haan, Germany), to obtain a chip size lower than 1 cm. Untreated, milled rapeseed straw contained (dry weight) 35.5% glucan; 18.5% xylan, 2.2% galactan, 1.0% arabinan, 1.2% mannan, 16.8% lignin, 2.6 acetyl groups; 5.3% ash and 13.1% extractives [2].

Sulfuric acid pretreatment of rapeseed straw was carried out in a 1 L reactor (Parr Instr. Co., IL, USA). 36 g of dried and milled rapeseed straw was suspended in 600 mL of aqueous sulfuric acid solution 0.5% (w/v) at 180 °C for 20 min; these conditions were previously optimized by Castro et al. [18] in terms of enzymatic hydrolysis yield. The time to reach the working temperature was 37 min while the cooling time until room temperature was 15 min. After pretreatment, liquid and solid phases were separated by vacuum filtration. The liquid fraction (prehydrolysate) was analyzed for sugars, acetic acid and sugar-degradation products such as furfural, 5-hydroxymethylfurfural (HMF) and formic acid. The solid fraction was washed several times with distilled water to eliminate acid solution, dried at 35 °C and analyzed for sugar and lignin content. This pretreated solid was used as substrate in the following assays of different process configurations (SHF, SSF and PSSF).

2.2. Enzymatic hydrolysis

The enzymatic hydrolysis based on commercial Cellic® CTec3 (Novozymes A/S, Denmark) was carried out in 100 mL Erlenmeyer flasks. Enzyme loading was 15 FPU/g substrate of Cellic® CTec3 supplemented with β-glucosidase (15 IU/g substrate). Tween-20 was added (0.1% v/v) in all assays to prevent the unproductive binding of the cellulases to the lignin residues, allowing more enzymes to be available for the conversion of cellulose [23]. Some authors have reported that the addition of Tween-80 in enzymatic hydrolysis increased cellulose digestibility of different lignocellulose substrates compared with those of the Tween-free process [24,25]. Enzymatic saccharification of pretreated solids was performed at 7.5%, 15% and 20% dry matter in the presence of 0.05 M sodium citrate buffer (pH 4.8). The flasks were incubated at 50 °C in an orbital shaker (Certomat-R, B-Braun, Germany) at 150 rpm for 72 h. Erlenmeyer flasks were withdrawn at 2, 4, 6, 10, 24, 48 and 72 h for glucose concentration measurements and compared to commercial cellulose (Sigmacell) controls with corresponding loading. All experiments were carried out in triplicate, the average results and standard deviations are shown. Additionally, blanks of the enzyme mixtures for each substrate loading were prepared and analyzed by HPLC in order to subtract the sugar content since the commercial enzymes contain glucose in monomeric and oligomeric form.

A fed-batch enzymatic hydrolysis configuration was assayed for comparing with batch configuration at 15% and 20% (w/v) solids loading. In the fed-batch enzymatic hydrolysis, batch process was firstly taken in the beginningwith 7.5% and 15% (w/v) solids loading. As the hydrolysis proceeded, the solid content of slurry decreased obviously. To increase the solids loading, 7.5% and 5% (w/v) fresh substrate, respectively, was fed at 24 h. Simultaneously Cellic® CTec3 was fed with 15 FPU/g substrate. The fed-batch enzymatic hydrolysis conditions were the same as in the batch hydrolysis.

2.3. Microorganism, medium and yeast cultivation

Saccharomyces cerevisiae (Fermentis ethanol red, France) was used for fermentation assays. Yeast inocula were prepared in glucose synthetic media consisting of (g/L): yeast extract, 5; $ClNH_4$, 2; KH_2PO_4, 1; $MgSO_4$ $7H_2O$, 0.3; and glucose, 30. Cells were grown in 100 mL Erlenmeyer flasks with a volume of 25 mL and agitated on a rotary shaker (Certomat-R, B-Braun, Germany) at 150 rpm and 30 °C for 24 h. For all experiments yeast inoculum used was 4% (v/v), corresponding to a cell addition of 0.25 g/L.

2.4. Process configurations

2.4.1. Separate hydrolysis and fermentation (SHF)

The slurries obtained after enzymatic hydrolysis were centrifuged at 10,000g for 10 min and the supernatants (glucose solution) subjected to fermentation for 24 h in an orbital shaker at 35 °C and 150 rpm after adjusting to pH 5 with 15 M NaOH. Fermentation tests were performed in 100 mL Erlenmeyer flasks containing 25 mL of fermentation medium with the nutrients described above for inoculum cultivation except for glucose, which was replaced with sugar solutions obtained after enzymatic hydrolysis. The flasks were equipped with a thick rubber stopper, through which one stainless-steel needle had been inserted to permit evolved CO_2 to leave and maintain microaerobic conditions. For all experiments the fermentation was started by inoculation of a cell suspension resulting in an initial cell mass concentration of 0.25 g/L. All experiments were carried out in triplicate and the average results are given. Ethanol and sugar concentrations were measured at the end of fermentation process, at 24 h. The quantity of potential ethanol produced from the glucose present in the enzyme solution was subtracted from the measured ethanol concentration in each case.