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Optimization of corn stover biorefinery for coproduction of oligomers and second generation bioethanol using non-isothermal autohydrolysis

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ABSTRACT

Corn stover was used for manufacturing 2nd generation bioethanol following a biorefinery scheme based on fractionation by autohydrolysis and further Simultaneous Saccharification and Fermentation (SSF) of pretreated solids. Autohydrolysis was performed under a wide range of severities to identify conditions leading simultaneously to a liquid phase containing hemicellulosic saccharides (accounting for up to 68% of initial xylan) and to a solid phase with high enzymatic susceptibility. SSF experiments were carried out under a variety of experimental conditions to assess the effects of the major operational variables. The glucan conversion into ethanol reached values up to 86%, with a bioethanol concentration of 37.8 g/L. Fed-batch operation in the SSF stage allowed the utilization of higher solid loadings, allowing an increase in the bioethanol concentration up to 51.6 g/L, or to reduce the amount of enzymes needed for reaching a given conversion.

1. Introduction

The increasing prices of fossil fuels and the environmental problems associated with their utilization have boosted the search for renewable energy sources. Recent studies have been focused on the biorefinery concept, looking for the sustainable production of a variety of fuels, chemicals and materials from biomass. Many governments consider biofuels as a strategic alternative to reduce both the dependence on non-renewable resources and the greenhouse gas (GHG) emissions. The U.S. Department of Energy (DOE) has committed to replace 30% of gasoline by biofuels by 2030, whereas the European Union has proposed 20% replacement of transportation fuels from fossil sources by others from renewable materials by 2020, in order to increase the security of supply and to reduce emissions of GHG.

Commercial biofuels are already a reality in several countries: for example, in Brazil and U.S., first generation bioethanol is produced at large scale from sugarcane and corn starch, respectively. However, the manufacture of biofuels from feedstocks suitable as food or feed entails a number of undesirable consequences, particularly the shortage of supply (and the concomitant increase in price) of basic foods. Because of this, many European countries have paid attention to the manufacture of biofuels from agricultural residues (Fischer et al., 2010). Some of the advantages of second generation bioethanol are as follows (Gnansounou, 2010): increased reduction in GHG emissions compared to the first generation bioethanol; possibility of using low-cost feedstocks; and geographical diversity of supply.

"Lignocellulosic materials (LCM) are more attractive feedstocks for bioethanol production than starchy materials or sugars, as these latter can be used as food of feed. However, LCM are difficult to process because of their heterogeneous and rigid nature. LCM contain three major polymeric components (cellulose, hemicelluloses, and lignin) interpenetrated in a three-dimensional matrix."

Bioethanol production from LCM may involve three major steps (Romaní et al., 2011; Wyman et al., 2005): (a) pretreatment of the raw material to increase its susceptibility to further processing (with the eventual generation of valuable byproducts); (b) enzymatic hydrolysis of cellulose to obtain sugars, and (c) biological conversion of sugars to ethanol.

Pretreatment is the most influential and expensive stage, and defines the rest of the technological process (Krishnan et al., 2010;

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Mosier et al., 2005; Romaní et al., 2010; Wyman et al., 2005). An optimal pretreatment should fulfill the following conditions (Gírio et al., 2010; Mosier et al., 2005; Sun and Cheng, 2002): (i) simple and economical operation; (ii) reduced operating costs (including energy, water and chemicals); (iii) ability to change the structure of LCM with small losses of polysaccharides; (iv) recovery of valuable compounds from hemicellulosic fraction; (v) generation of small amounts of unwanted degradation products (such as furfural and hydroxymethylfurfural); (vi) ability to produce solids with high cellulose content and high susceptibility to enzymatic hydrolysis; (vii) ability to produce high quality lignin (or lignin-containing byproducts) with small amounts of wastes.

Autohydrolysis is a technology suitable for LCM pretreatment that allows the depolymerisation of hemicelluloses, leading to the formation of soluble saccharides as major reaction products. When the raw material is rich in xylan (a polymer made up of xylopiranose units), the autohydrolysis conditions can be tuned to obtain xylooligosaccharides (XO) as the main reaction products (Vázquez et al., 2005). The xylan backbone may contain a number of substituents, including arabinosyl units, acetyl groups and uronyl residues (Parajó et al., 2004).

The fermentative production of ethanol from LCM can be carried out by consecutive stages of hydrolysis and fermentation, or in single stage of Simultaneous Saccharification and Fermentation (SSF). In this latter, enzymes and fermenting microorganisms are present in the same medium. The major SSF advantages are as follows (Sun and Cheng, 2002; Balat, 2011): (i) the glucose that is being generated from cellulose by enzyme-catalyzed hydrolysis is simultaneously being converted into ethanol by the microorganisms, reducing the substrate inhibition of enzymes; (ii) the enzyme loadings are reduced respect two-step operation; and (iii) higher product yields can be achieved.

Saccharomyces cerevisiae is the yeast of choice in the ethanol industry because of its many advantages, including favourable efficiency and conversion rate, high ethanol yield from glucose, and high resistance to ethanol (Hasunuma and Kondo, 2012; Van Eylen et al., 2011). When fermentation is performed in batch mode, the microorganism works in high substrate concentration initially and at a high product concentration finally (Balat, 2011; Chandel et al., 2007). Operating in fed-batch mode, the microorganism works at a lower substrate concentration along fermentation, while ethanol accumulates in the medium (Chandel et al., 2007; Sánchez and Cardona, 2008).

This work provides an experimental assessment on the production of second generation bioethanol from hydrothermally pretreated corn stover using two different approaches: batch and fed–batch SSF, using autohydrolyzed corn stover as a substrate.

2. Materials and methods

2.1. Raw material

Corn stover was collected from a local plantation (Ourense, North West of Spain), milled to pass an 8 mm screen, air-dried, homogenized in a single lot to avoid differences in composition, and stored in a dark and dry place until use.

2.2. Analysis of the raw material

Raw material was analyzed for moisture (TAPPI T 264 cm-07), ash (TAPPI T 211 om-12), extracts (TAPPI T 264 cm-07), and subjected to two-step quantitative acid hydrolysis (QAH) (TAPPI T 249 cm-09). The liquid phase resulting from QAH was filtered through 0.45 μm membranes and assayed for glucose, xylose, arabinose and acetic acid by HPLC using an Agilent Technologies 1100 Series instrument with Refractive Index Detector and a BioRad Aminex HPX-87H column. The results allowed the determination of glucan, xylan, arabinan and acetyl groups present in the raw material. The solid residue resulting from QAH was considered as Klason lignin. Table 1 shows the composition of the raw material.

Table 1
Corn stover composition (g/100 g raw material, on dry basis, based in 4 replicates).

Composition	Content	Standard deviation
Glucan	34.48	0.14
Xylan	14.54	0.07
Arabinan	2.16	0.06
Acetyl groups	1.93	0.02
Klason lignin	18.49	0.21
Extractives	12.20	0.41
Ash	5.00	0.03
Proteins	4.25	0.22

2.3. Corn stover pretreatment

Corn stover was subjected to non-isothermal autohydrolysis using a pressurized reactor with an internal volume of 3.75 L (Parr 4842 model no. 4551, Parr Instruments Company, Moline, Illinois, USA), equipped with a four-blade rotor, heated by an external mantle and cooled by flowing water through an inner loop. The medium was agitated at 150 rpm. Corn stover and water were mixed at a liquid to solid ratio (LSR) of 10 g liquid/g oven-dry raw material, heated to reach the desired maximum temperature (T_{MAX}, in the range of 180–223 °C) and cooled immediately.

The intensity of treatments can be comparatively measured in terms of "severity" (S_0), defined as the logarithm of the severity factor R_0 (Lavoie et al., 2010). S_0 measures the combined effects of time and temperature along the heating and cooling periods, and was calculated using the equation:

$$S_0 = \log R_0 = \log\left[R_{0\,\mathrm{HEATING}} + R_{0\,\mathrm{COOLING}}\right] = \log\left[\int_0^{t_{MAX}} \exp\left(\frac{T(t) - T_{REF}}{\omega}\right) dt + \int_{t_{MAX}}^{t_F} \exp\left(\frac{T'(t) - T_{REF}}{\omega}\right) dt\right] \quad (1)$$

where: t_{MAX} is the time (in min) needed to achieve maximum temperature (T_{MAX}, °C); t_F is the time (in min) required for the entire heating-cooling cycles; $T(t)$ and $T'(t)$ (°C) are the temperature profiles in heating and cooling, respectively, and ω and T_{REF} are parameters whose values have been reported in literature (ω = 14.75 °C; T_{REF} = 100 °C).

Once the reactor was cooled, the solid and liquid phases were separated by filtration for further analysis.

2.4. Analysis of liquid and solid phases from autohydrolysis

Aliquots of the liquid phase from autohydrolysis media were filtered through 0.45 μm membranes and used for direct HPLC determination of monosaccharides (glucose, xylose, arabinose), acetic acid, HMF and furfural. Other aliquots were assayed for non-volatile compounds (*NVC*) by drying at 105 °C until constant weight. Finally, other aliquots were subjected to quantitative posthydrolysis with 4% w/w sulphuric acid at 121 °C for 40 min to convert oligomeric saccharides into monosaccharides, and the resulting hydrolyzates were filtered through 0.45 μm membranes and analyzed for monosaccharides, acetic acid, HMF, furfural, and monosaccharides using the same HPLC method indicated above. The increases in the concentrations of glucose, xylose, arabinose and acetic acid respect to the raw autohydrolysis liquor measured the content of glucosyl, xylosyl, arabinosyl and acetyl moieties