Fermentable Sugars Production from Acid-Catalysed Steam Exploded Barley Straw

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Barley straw is being considered a potential lignocellulosic raw material for fuel-ethanol production. Ethanol production from lignocellulosic biomass consists in three main steps: pretreatment, enzymatic hydrolysis and fermentation. In order to improve the accessibility of the enzymes during enzymatic hydrolysis, a dilute phosphoric acid steam explosion pretreatment was applied. A three factor experimental design with temperature (160-200 ºC), residence time (10 – 30 min) and phosphoric acid concentration (1 – 3 % w/v) as relevant factors was performed after a previous stage of overnight soaking. Once the pretreatment was done, both liquid and pretreated solids were separated by filtration and analysed. The pretreated solids were further subjected to enzymatic hydrolysis (S/L= 5 %, 50 ºC, 150 rpm, 15 FPU / g substrate, pH = 4.8) and the released glucose was determined. Optimal pretreatment conditions were determined based on highest recovery of hemicellulosic sugars and minimum inhibitors in the liquid fraction, and highest concentration of glucose after enzymatic hydrolysis of the solid residue. The best operational conditions were found to be 160 ºC pretreatment temperature, 30 min residence time and 2.88 % phosphoric acid concentration, which resulted in the recovery of 44.0 % of xylose in the liquid fraction together with 7.5 % of the initial glucose. The enzymatic hydrolysis of the pretreated solid in these conditions resulted in the release of 61.0 % of the glucose contained in the raw material.

1. Introduction

Current depletion of traditional energy sources will result in a future shortage of energy and materials. This fact has attracted attention towards renewable sources, second generation bioethanol being one of the most promising options (Vargas et al., 2015). Ethanol production from biomass is of particular interest to achieve energy security (García-Aparicio et al., 2011), reduce greenhouse-gas emissions and dependence on imports (Vargas et al., 2015).

Lignocellulosic biomass is an interesting alternative to traditionally used sugar or starch biomass due to the huge amount available, no competition with food or feed production and low cost. Lignocellulosic materials can derive from multiple sectors, a remarkable one being the agricultural sector in which productive process residues are predicted to lead future biorefinery industry (García-Aparicio et al., 2007). Among them, barley straw is being taking into consideration as a good candidate lignocellulosic raw material for ethanol production. Straw is the main byproduct obtained after barley harvesting with an average production of 0.53 kg straw/kg grain; just in the case of Spain, barley fifth largest world producer, barley straw accounts for up to 4.5 million t/y (Vargas et al., 2015). Currently, most of barley straw is burned, discarded or used as feed with low nutritional value and digestibility. All of this along with its high carbohydrate content makes barley straw an abundant, cheap renewable energy source for ethanol production (Han et al., 2013).

Due to high stability of lignocellulosic materials, a pretreatment step is necessary in order to remove structural obstacles and compounds that affect enzymatic hydrolysis effectiveness (Shang et al., 2017). This enhances the rate of enzymatic digestibility of cellulose by allowing a better contact between enzymes and biomass cellulose resulting from an increment in the substrate pore size as a consequence of removing hemicellulose fraction (Vargas et al., 2015). In this stage, limiting formation of degradation products is essential to avoid sugars

fermentation inhibition (Persson et al., 2009). Steam explosion (SE) is a physical-chemical process and one of the most effective pretreatment techniques for the disruption of lignocellulosic biomass structure leading to lignin barrier disruption and hemicellulose removal (Erdocia et al., 2017). SE presents numerous advantages such as low use of chemicals, low energy demand, efficient biomass disruption characteristics for agricultural residues and hardwoods and it is already available on a commercial scale (García-Aparicio et al., 2007). It can be performed on its own, or in acid or alkaline environments. Acid environment hydrolyses cellulose as well as hemicelluloses (Persson et al., 2009). In spite of its higher cost compared with other acids such as sulfuric or hydrochloric acids, phosphoric acid has several advantages like being less corrosive and less toxic, representing a reduction in the cost of plant construction. Moreover, at low concentrations, phosphoric acid presents a high capacity of solubilizing the hemicellulose fraction with the advantage of being a source of phosphorus, a nutrient for the microorganism in the fermentation of prehydrolysates. However, to our best knowledge, phosphoric acid has never been used as catalyst in steam explosion pretreatment of barley straw. Hence, the aim of this work was to assess the best operational conditions considering the highest sugar production along with lowest generation of inhibitors.

2. Materials and methods

2.1 Raw material

The barley straw was supplied by a local farmer of Jaén (southern of Spain). The air-dried barley straw was milled using a laboratory hammer mill (Retsch, SM2000) with an approximate particle size of 1 cm and stored at room temperature. The moisture and the composition of the raw material was determined according to the standard procedures of the National Renewable Energy Laboratory of USA (NREL, 2012).

2.2 SE pretreatment

A mass of 90 g (dry weigt) of barley straw was soaked in 2L of phosphoric acid solution overnight. The impregnated straw was then filtered and the moisture was measured in a moisture analyzer (MA 50/1.R, Radwag). The straw was pre-treated in a steam explosion pilot unit based on Masonite technology (Galbe and Zacchi, 2012) with a 4.5-L reaction vessel. After pretreatment, the solid and liquid were separated by filtration. The solid was washed until neutral pH, dried in the oven at 38 ºC, and stored in plastic bags at room temperature. The liquid, also referred to as prehydrolysate, was analysed for sugars and other compounds.

2.3 Experimental design

Barley straw was SE pretreated at different operational conditions according to a three factor experimental central composite design (CCD), with a total of 20 experiments including five replicates at the center of the domain selected for each factor, i.e. temperature (160 - 200 ºC), residence time (10 - 30 min) and phosphoric acid concentration (1 - 3 % w/v), as shown in Table 1. The factor α is included to maintain the rotatability of the experimental design (α=1.68). In addition, three runs were performed to verify results calculated at optimal conditions according to the model. Experiments were performed in random order. The experimental data were analysed by the statistical software Design Expert 7.0, StatEase Inc., Minneapolis, USA.

2.4 Analytical methods

The pretreated solid fractions were analysed for cellulose and hemicellulosic sugars and lignin content (NREL, 2012). Inhibitory compounds (acetic acid, formic acid, furfural and hydroxymethylfurfural, HMF) were determined by HPLC, Agilent Technologies 1260 model (Santa Clara, CA, USA) with Aminex HPX-87H column (Bio-Rad Hercules, CA, USA) operating at 65 °C with 5 mM H_2SO_4 solution as eluent and operating flow of 0.6 mL/minute). Oligomeric sugars in the liquids were determined by a post-hydrolysis, with 3 % H_2SO_4 w/v at 120 °C for 30 min, comparing the sugar content before and after the post-hydrolysis.

2.5 Enzymatic hydrolysis test

The washed solid fractions obtained after pretreatment were used as substrate for enzymatic hydrolysis by a cellulolytic complex kindly provided by Novozymes A/S (Denmark). Cellulase enzyme (Cellic Ctec2, Bagsvaerd, Denmark) loading was 15 Filter Paper Units (FPU)/g substrate. Fungal β-glucosidase (Novozym 50010, Novozymes A/S) was used at an enzyme loading of 15 International Unit (IU)/g substrate. Enzymatic hydrolysis was performed in 0.05M sodium citrate buffer (pH 4.8) at 50 º 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 in the HPLC.

Table 1: Experimental design for SE pretreatment of barley straw. Data in parentheses correspond to coded values

Run	Temperature (º C)	Time (min)	Phosphoric acid concentration (w/v)
1	146.36 (-α)	20 (0)	2 (0)
2	180 (0)	20 (0)	3.68 (+α)
3	180 (0)	20 (0)	0.32 (-α)
4	160 (-1)	10 (-1)	3 (+1)
5	200 (+1)	30 (+1)	3 (+1)
6	180 (0)	20 (0)	2 (0)
7	200 (+1)	30 (+1)	1 (-1)
8	180 (0)	20 (0)	2 (0)
9	200 (+1)	10 (-1)	1 (-1)
10	180 (0)	20 (0)	2 (0)
11	160 (-1)	30 (+1)	1 (-1)
12	180 (0)	20 (0)	2 (0)
13	180 (0)	20 (0)	2 (0)
14	160 (-1)	30 (+1)	3 (+1)
15	180 (0)	20 (0)	2 (0)
16	180 (0)	3.18 (-α)	2 (0)
17	180 (0)	36.82 (+α)	2 (0)
18	213.64 (+α)	20 (0)	2 (0)
19	200 (+1)	10 (-1)	3 (+1)
20	160 (-1)	10 (-1)	1 (-1)

3. Results and discussion

3.1 Raw material

The composition of the barley straw is summarized in Table 2 and agrees with that reported by other authors (Sáez et al., 2013). The total sugar fraction of the dry biomass was 74.52 %. Glucose was the major component (42.27 %), and xylose, which was the major hemicellulose constituent, was 30.23 %.

Table 2: Composition of the barley straw, % dry weight.

Glucose	Xylose	Arabinose	Galactose	Acid Insoluble Lignin	Acid Soluble Lignin	Extractives (glucose)
42.27±1.75	30.23±0.16	1.59±0.24	0.43±0.04	14.23±1.40	2.03±0.40	18.57±0.54 (2.53 ± 0.03)

3.2 Composition of the water insoluble solid (WIS) after steam explosion pretreatment.

After SE pretreatment, average solid recovery (expressed as WIS remaining after pretreatment divided by 100 g of raw material) was about 43 %. Reduction in WIS was mainly due to solubilisation and degradation of hemicellulose and extractives. After SE, the biomass composition changed because of the thermal degradation mainly of the hemicellulose component. Cellulose content in WIS increased in relation to untreated material ranging from 52 to 62 %, depending on the different pretreatment conditions. The greatest cellulose content was 74.18 + 3.66 g glucose/100 g raw material, obtained at 160 °C during 10 min and 3 % w/v of phosphoric acid (run 4). High temperature pretreatment (213°C) produced lower cellulose content in the pretreated material (57.24 %) due to slight cellulose solubilisation (run 18). At increased pretreatment severity, a decrease in the hemicellulose content was also observed. The chemical composition confirmed that the matter loss primarily occurs at the expenses of hemicellulose, the component being more thermally degradable.

3.3 Effect of pretreatment conditions on the composition of the liquid fractions

The composition of the liquors obtained from pretreatment was dependent on the experimental conditions. SE caused autohydrolysis reactions by which part of the hemicellulose and lignin were converted into soluble compounds (García-Aparicio et al., 2006). Regarding carbohydrates, xylose was the most abundant sugar in the liquid, where 50.59 % of the xylose initially present in the raw material was found; the values of glucose recovery were below 12 % of the glucose in raw material. Figure 1 shows the surface obtained from the experimental results when the concentration of xylose found in the liquors is adjusted as a function of the pretreatment factors. As can be seen, increasing concentration of phosphoric acid in the soaking step produces

an increase of the xylose concentration recovered in the liquor, at low levels of temperature, i.e., below 180ºC (Fig. 1a, at pretreatment time = 20 min). The process time seems to be the less relevant factor (Fig. 1b).
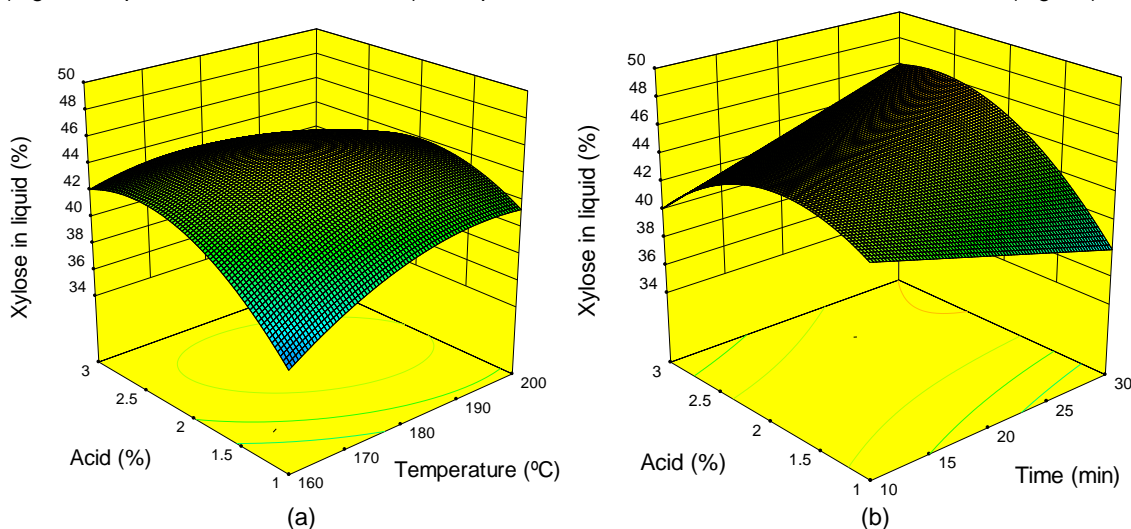

Figure 1: Response surfaces obtained from experimental results showing the effect of pretreatment factors on the concentration of xylose found in the liquid fractions obtained after pretreatment. a) Pretreatment time = 20 min b) Pretreatment temperature = 180 ºC.

In addition to sugars, other compounds such as acetic acid, formic acid, furfural and HMF were also present in the prehydrolysate, as shown in Table 3. All these products have been previously identified in other herbaceous biomass (Martín et al., 2002). The quantification of furfural can hardly explain hemicellulose losses during pretreatment. It is likely that hemicellulose was lost through volatilization of furfural (Cara et al., 2006). All these compounds have been described as potential inhibitors of yeast fermentation (Jönsson and Martín, 2016).

Table 3: Inhibitors concentration (g/L) in liquid fractions obtained after steam explosion pretreatment.

Run	Acetic acid	Formic acid	Furfural	HMF
1	0.11	0.11	0.04	0.00
2	0.19	0.16	0.51	0.03
3	0.26	0.23	0.33	0.00
4	0.13	0.13	0.07	0.00
5	0.08	0.12	0.90	0.07
6	0.20	0.20	0.71	0.11
7	0.17	0.16	1.25	0.17
8	0.14	0.14	0.36	0.00
9	0.21	0.21	1.03	0.12
10	0.19	0.18	0.52	0.03
11	0.11	0.11	0.08	0.00
12	0.17	0.16	0.53	0.03
13	0.22	0.22	0.64	0.10
14	0.13	0.13	0.17	0.00
15	0.18	0.18	0.60	0.04
16	0.23	0.23	0.20	0.04
17	0.18	0.17	0.93	0.11
18	0.16	0.16	0.93	0.11
19	0.20	0.20	0.00	0.03
20	0.10	0.11	0.04	0.00

3.4. Effect of the pretreatment conditions on the production of glucose from enzymatic hydrolysis of WIS

The main objective of pretreatment is to alter the structure of the fibres in order to increase the accessibility of enzymes to cellulose. Figure 2 shows how pretreatment conditions affect the amount of glucose released from the pretreated solids. In general, the enzymatic hydrolysis yield increased as the temperature did. The highest glucose production by enzymatic hydrolysis was obtained at 180 º C and 200 º C experiments, reaching near 70% of the initial glucose in the raw material. The enzymatic hydrolysis of untreated raw material resulted in only 26 % theoretical glucose yield in tests performed in parallel with pretreated substrates. The concentration of the phosphoric acid was less relevant than temperature, while the pretreatment time showed no effect.
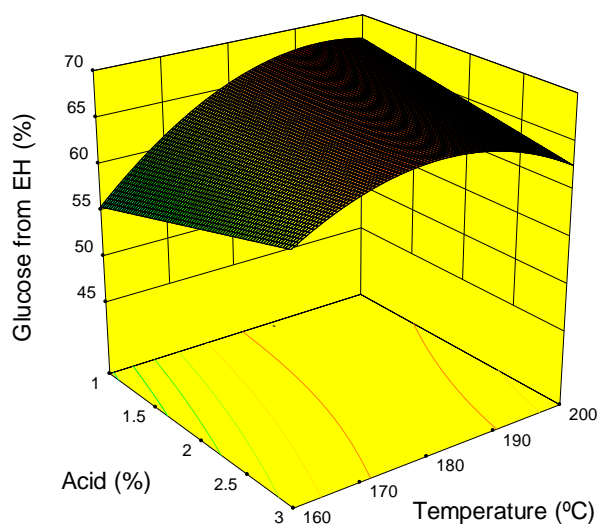

Figure 2: Glucose released from enzymatic hydrolysis of the barley straw pretreated solids as a function of acid concentration and temperature used for SE pretreatment.

3.5. Optimal conditions of pretreatment

To determine the best operational conditions of pretreatment, the optimization criterion was initially selected as the highest concentration of all fermentable sugars, considering glucose generated from pretreated solids by enzymatic hydrolysis, and also the hemicellulose-derived sugars, mainly xylose, that are liberated to the liquid fractions as a consequence of the pre-treatment. These sugars can also be directed to ethanol production using a pentose-fermenting microorganism (Díaz et al., 2009). Nevertheless, the presence of potential inhibitors in the liquid fractions should also be taken into account. For this reason the final criterion for selecting optimal pretreatment conditions was the combined maximization of the concentrations of glucose (from enzymatic hydrolysis) and xylose (in the liquids) along with the minimum degradation of both sugars. The best results were obtained when the hydrothermal pre-treatment was performed at 160.0 ºC for 30 min and 2.88 w/v phosphoric acid concentration. These conditions were carried out in a triplicate experiment (runs 21, 22 and 23) and the average results are shown in Table 4. Under such conditions, 61 % of the glucose initially present in the raw material was obtained from enzymatic hydrolysis, while 43.97 % of the initial xylose was recovered in the liquid (in addition to 7.49 % of the soluble glucose, that is, not coming from enzymatic hydrolysis of cellulose).

Table 4: Enzymatic hydrolysis yield (Y_{EH}, g glucose/100 g structural glucose of raw material), recovery of sugars (g/100 g of sugar in raw material) and inhibitors (g/L) in liquid fraction obtained at the optimal conditions of pretreatment

Run	Solid Y_{EH}	Liquid Glucose	Xylose	Acetic acid	Formic acid	Furfural
21	58.47	7.28	43.50	0.13	0.49	0.02
22	62.35	7.90	45.84	0.15	0.77	0.02
23	62.18	7.30	42.56	0.15	0.69	0.03
Average	61.00 ± 2.19	7.49 ± 0.35	43.97 ± 2.19	0.14 ± 0.01	0.65 ± 0.14	0.02 ± 0.01

4. Conclusions

Phosphoric acid catalysed steam explosion can be effectively used as a pretreatment of barley straw. The optimal conditions for this process can be determined by maximizing the glucose released from enzymatic hydrolysis of the pretreated solids and the sugars obtained in the liquid fractions and minimising at the same time the inhibitors present in the liquors. Using this optimization criterion, the best pre-treatment conditions proved to be 160.0 ºC, 30 min and 2.88 % w/v of phosphoric acid, resulting in a 61.0 % of the potential glucose release in the solid fraction and 44.0 % of the potential xylose in the liquid fraction. In addition, 7.5% of the initial glucose is also found in the liquids. Further work will be focused on the fermentation of these sugars, under different configurations.

Acknowledgements

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