OPTIMIZATION OF INTEGRATED ALKALINE-EXTRUSION PRETREATMENT OF BARLEY STRAW FOR SUGAR PRODUCTION BY ENZYMATIC HYDROLYSIS

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Abstract

In this work, an integrated one-step alkaline-extrusion process was tested as pretreatment for sugar production from barley straw (BS) biomass. The influence of extrusion temperature (T) and the ratio NaOH/BS dry matter (w/w) (R) into the extruder on pretreatment effectiveness was investigated in a twin-screw extruder at bench scale. A 2^3 factorial design of experiments was used to analyze the effect of process conditions [T: 50-100ºC; R: 2.5-7.5% (w/w)] on composition and enzymatic digestibility of pretreated substrate (extrudate). The optimum conditions for a maximum glucan to glucose conversion were determined to be R=6% and T=68ºC. At these conditions, glucan yield reached close to 90% of theoretical, while xylan conversion was 71 % of theoretical. These values are 5 and 9 times higher than that of the untreated material, which supports the great potential of this one-step combined pre-treatment technology for sugar production from lignocellulosic substrates. The absence of sugar degradation products is a relevant advantage over other traditional methods for a biomass to ethanol production process since inhibitory effect of such product on sugar fermentation would be prevented.

Keywords: lignocellulose, experimental design, extrusion, enzymatic hydrolysis, alkaline treatment

Introduction

The increasing global energy demand, which relies mostly upon the dependence on fossil fuels and a raising concern about the greenhouse gases emissions have lead to a search for new and sustainable energy sources. In this context, biofuels represent a solid alternative to conventional fossil fuels. Within this field, ethanol produced from lignocellulosic biomass is considered a key element to boost implementation of bioethanol in the current fuel market since it avoids the most important drawback of the first generation bioethanol: its competition with food crops. However, since the structure of these materials makes them very recalcitrant to the enzymes accessibility [1], a pretreatment is needed to break down the lignin net and disrupt the crystalline structure of cellulose, increasing the surface area and porosity of the biomass fibres.

Among the several pretreatments that are being currently studied and further developed, extrusion stands out for its ability to provide high shear, rapid heat transfer, and effective and rapid mixing [2]. Other advantages of this method are the feasibility of continuous operation and its versatility to adopt different process configurations. In addition, extrusion can be run at moderate temperature, which is advantageous in comparison to other hydrothermal pretreatments, since the formation of inhibitory byproducts as 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde (hereinafter referred as furfural and HMF) can be prevented.

Pretreatment of different biomasses by extrusion alone or in combination with different chemicals and additives for sugar production by enzymatic hydrolysis has been reported by several authors during the last years. The performance of extrusion and the influence of the operation parameters has been studied on switchgrass, prairie cord grass, corn stover and more recently on pine wood chips by Karunanithy and Muthukumarappan [2, 3, 4, 5, 6], while other researchers have focused extrusion pre-treatment on biomasses such as *Miscanthus sp* [7], Douglas fir [8], soybean hulls [9], rice straw [10] and a combination of wheat bran and straw [11].

Lately, extrusion has been also pointed out as an interesting technique to be used together with other pretreatments, in a two-step process strategy. For example, Lee et al. [12] used extrusion as complementary step for Douglas fir after hot-compressed water treatment. Results showed sugar yields 5 times higher than without passing through the extruder and a fine fibrous morphology on a sub-micro/nanoscopic scale. The combination of extrusion and diluted acid pretreatment has been tested on rice straw and proved to be an effective method to maximize hemicellulose hydrolysis and enhance glucan to glucose conversion by enzymes [13]. More recently, the conditions for sequential treatment of corn stover by extrusion and surfactant pretretament has been optimized [14], aimed at increasing efficiency of hydrolysis for bioethanol production.

On the other hand, mild alkaline pretreatment is a well-known method to enhance the enzymatic digestibility of the lignocellulosic biomass. It is generally more effective in the pretreatment of agricultural residues and herbaceous crops [15]. Biomass soaking in sodium hydroxide, potassium hydroxide, ammonia or lime, in a concentration below 2%, has been reported to cause delignification, xylan loss, decrease of cellulose crystallinity and swelling of biomass [15, 16, 17]. As a consequence, sugar production increases as reported by McIntosh and Vancov [18], who obtained 5.6-fold higher sugar yields by pretreating sorghum straw in 2% NaOH at 121 ºC for 60 min.

The combination of both extrusion and alkaline pretreatment has been explored in some recent works by Lamsal et al. [19] and by Karunanithy and Muthukumarappan [4], with different results. Lamsal could not find any improvement in sugar yield by soaking wheat bran and soybean hull in a solution of sodium hydroxide, urea and tiourea (10% w/w) and then introducing the mixture in a twin-screw extruder at 7 Hz and maximum barrel temperature of 150 ºC, compared to a simple grinding of the biomass. However, the extensive washing of the pretreated substrate took away the solvents and enzymatic inhibitors, resulting in enhanced sugar yields of 60 - 73% and 25 - 36%, respectively, for wheat bran and soybean hull. On the other hand, Karunanithy and Muthukumarappan optimized the extrusion performance for prairie cord grass at 114 ºC, 122 rpm, 1.70 % NaOH concentration and 8 mm particle size, reaching a maximum glucose and xylose recovery of 86.8 and 84.5 % respectively, after enzymatic hydrolysis. These authors claim that the low alkaline concentration used allow hydrolysing the extruded material without washing of the biomass.

In both works, the alkali soaking was a previous step to the extrusion and was done in a discontinuous way. The aim of this study is to integrate both processes in a single step to pretreat barley straw, enabling a continuous operation of the whole pretreatment, reducing the contact time between the NaOH and the substrate and possibly improving the effect of the alkali by a thoroughly mixing in the extruder. The final purpose of the present work is to optimize the operation conditions, namely the ratio NaOH/barley straw dry weight (w/w) (R) and extrusion temperature (T), in an integrated alkaline-extrusion pre-treatment by using a statistical experimental design, in order to enhance glucan and xylan digestibility by further enzymatic saccharification.

Material and Methods

Raw material

Barley straw (6% moisture content) was provided by Centre for the Development of Renewable Energy Sources (CEDER), (Soria, Spain). Biomass was coarsely crushed to about 5 mm particle size using a laboratory hammer mill (Retsch), homogenised and stored until used.

Extrusion pretreatment

Extrusion was performed in a twin-screw extruder (Clextral Processing Platform Evolum® 25 A110, Clextral, France), composed of 6 modules of 100 mm length each (Figure 1). In module 5 a filtration step was set up in order to separate liquid from solid fraction (filtrate and extrudate respectively) after extrusion. The modules have a heating and cooling system that allows setting a temperature profile throughout extrusion process. The temperature set for the module is considered to be the extrusion temperature (T). The screws diameter is 25 mm and they were configured to produce transport, mixing and shearing effects along the process, as depicted in Fig. 1. Two metering pumps connected to the extruder are used to supply the catalyst (NaOH solution at 10% w/v) and H_2O to the process. Biomass feeding was done through a volumetric feeder KMV KT20 (Ktron), which has a flow capacity up to 1.2 kg/h for 5 mm barley straw. The feeder screw speed rate was set to provide a continuous feed rate of 0.6 kg/h.

Operating conditions were set to achieve moderate values of R, between 2.5 and 7.5 % (w/w), and temperature, between 50 and 100ºC. Based on preliminary experiments, a fixed motor speed of 150 rpm was used for all runs. At this condition, the residence time of the biomass inside the extruder is about 2 min.

After extrusion, solid extruded material was recovered and washed thoroughly with distillate water until neutral pH (hereinafter, extrudate). Filtrate was also collected and analyzed for sugar and degradation compounds, i.e. furfural and HMF.

A portion of extrudate was dried and analyzed for carbohydrates and lignin composition to evaluate changes in comparison to untreated BS. Samples were stored at 4ºC in hermetic plastic bags until use in enzymatic hydrolysis experiments.

Experimental design

In order to study the variation of extrudate composition and enzymatic digestibility against selected process variables and to determine the optimum extrusion conditions leading to a maximum enzymatic digestibility in extrudate, a 2^3 factorial experimental design with two variables [ratio NaOH/barley straw dry weight (w/w), R and extrusion temperature, T] was employed. The design resulted in 9 runs (Table 1) and was developed with StatGraphics Plus 5.0 Enterprise Edition (Satistical Graphics Corporation, Princenton, NY). The order of experiments was randomized, as a way to avoid the effect of lurking variables. The levels of optimized variables were 2.5 – 5 – 7.5 % for R and 50 – 75 – 100 ºC for T. They were selected according to the criteria of using mild conditions and based on preliminary extrusion trials on BS.

Enzymatic hydrolysis

The extrudate was used as substrate for enzymatic hydrolysis (EH) in 0.05 M sodium citrate buffer (pH 4.8) at 50ºC and 5% (w/v) dry extrudate load. As a control, untreated barley straw was also subjected to enzymatic hydrolysis at the same conditions. Experiments were performed in 100 ml Erlenmeyer flasks on a rotary shaker (Certomat-R B-Braun, Germany) at 150 rpm. Enzymatic cocktail consisting of commercial cellulase boosted with commercial xylanase in a proportion 9:1 in protein content was added in a dosage of 10 mg protein (15 FPU of cellulase)/g dry extrudate. The supplementation with xylanase was aimed at promoting xylan hydrolysis, based on the significant xylan content of extrudates after extrusion (see below). The enzymes were kindly provided by Novozymes A/S (Denmark). After 72 h saccharification, glucose and xylose concentration in EH media was measured by HPLC as described below in analytical methods section.

The parameter used to evaluate the hydrolysis performance is the enzymatic hydrolysis yield (EHY), which is defined as the glucose/xylose released during EH divided by the potential glucose/xylose (calculated based on glucan/xylan content of the solid extrudate), and expressed as percentage.

Raw and extruded biomass characterization

National Renewable Energy Laboratory (NREL, CO) laboratory analytical procedures (LAP) for biomass analysis [20] were used to determine carbohydrates, acid-insoluble lignin, acid-soluble lignin, acetyl groups, extractives and ash content in raw material. Extrudates were analyzed for carbohydrates and acid-insoluble lignin by the same procedures.

Analytical methods

The filtrate was recovered after extrusion and analysed for its content of monomeric and oligomeric sugars. The oligosaccharides ratio was determined as the difference in monomeric sugar concentration before and after mild acid hydrolysis (3% v/v H_2SO_4, 120 ºC and 30 min). Sugars were analysed by high-performance liquid chromatography (HPLC) in a Waters 2695 liquid chromatograph with refractive index detector, as described in Cara et al [21]. Likewise, glucose and xylose concentration after completion of enzymatic hydrolysis tests was measured in EH media by HPLC using the same column. Furfural and HMF were analyzed by HPLC (Hewlett Packard, Palo Alto, CA), using an Aminex ion exclusion HPX-87H cation- exchange column (Bio-Rad Labs, Hercules, CA) at 65ºC. Mobile phase was 89% 5 mM H_2SO_4 and 11% acetonitrile at a flow rate of 0.7 mL/min. Column eluent was detected with a 1040A Photodiode-Array detector (Agilent, Waldbronn, Germany).

Results and discussion

Raw Material Composition

Table 2 presents the results of barley straw composition. The dry matter distribution shows an average value of 39.1 % cellulose, 25.7 % hemicellulose and 15.2 % lignin, making it a very promising substrate for bioconversion to ethanol after a suitable pre-treatment based on high total carbohydrate content of 65% on dry weight basis (dwb). The degree of lignification of barley straw biomass is in the range of that reported for other agricultural residues such as wheat straw (17%) [22] or corn stover (17-19%) [23]. A significant fraction of the feedstock, about 10%, is made up of both water and ethanol soluble materials, included in the term extractives. Water extract was analyzed for sugar content and glucose was found in concentration about 2g /l (equivalent to about 1 g/100 g dw biomass). Minor contents of other sugars were found. The high ash content of barley straw (6.8 %) is consistent with the presence of silica as a major mineral component of cereal straws. In general, results are comparable to the ones reported by Linde et al. [24]; Persson et al. [25]; García-Aparicio et al. [26] and Li et al. [27] for raw barley straw.

Extrusion pretreatment

Effect of extrusion conditions in extrudate and filtrate composition

In order to evaluate the efficiency of extrusion as a pretreatment to fractionate barley straw biomass and so affect the enzymatic digestibility of the extruded material, changes in the composition of extrudates with respect to the raw material were measured at the different extrusion conditions. Moreover, filtrate fraction was analyzed for sugar composition and the presence of furfural and HMF. Results are shown in Table 3.

Regarding the results of biomass composition variation because of extrusion (Table 3), in general alkali concentration seems to exert a greater effect than temperature, in the range of conditions tested. To evaluate changes in comparison to raw BS, it has been considered that extractives and ash are solubilized into the filtrate during extrusion (these components are not found in extrudate composition analysis) and that so, cellulose, hemicellulose and lignin remaining in the extrudate would be concentrated at least by a factor corresponding to the sum of extractives and ash content, which accounts for 17% of BS dry weight. So, to refer composition values shown in Table 1 to an “extractives and ash free” basis BS, they can be multiplied by a factor of 1.2 [100/ (100-17)]. Taking into account these considerations, it can be seen that at R of 2.5%, extrudate composition at the three temperatures tested is similar to that expected if raw BS composition data are multiplied by the concentration factor above, i.e., 47% cellulose, 25.4% xylan and 18.2% lignin, indicating very slight or no destructuration of major components in BS at these conditions.

Particularly for hemicellulose fractionation, the one- step alkaline-extrusion process, in the conditions tested in the present work, does not exert an impact in hemicellulose breakdown at R of 2.5 and 5% and only at 7.5% for all temperature conditions (runs 3, 6 and 9), xylan content values are below than expected if the concentration factor is considered (25.4%), indicating hemicellulose solubilization. Even when xylan solubilization occurs at the highest alkali ratio, a significant part of the xylan in raw material is remaining in extrudate after extrusion, which could be hydrolyzed in the subsequent enzymatic hydrolysis step by specific enzymes.

Sugar analysis of the prehydrolysate (Table 3) shows negligible concentrations of glucose (data not shown) and varying amounts of hemicellulose-derived sugars, mostly xylose and arabinose and in minor concentration galactose and mannose (data not shown). Increasing hemicellulose-derived sugar content is found as R raises, reaching concentrations up to 18 g/l at 75 ºC and 7.5% R (Table 3). It was found that sugars measured in filtrate were mostly in oligomeric form in all R conditions, regardless the extrusion temperature. It is important to highlight that neither furfural nor hydroxymethyl furfural (HMF) was detected in the filtrates.

The effect of exposure to alkaline substances during the pre-treatment process on hemicellulose loss is well supported in the literature in experiments at different operation conditions for alkali treatment. McIntosh and Vancov [28] obtained 18.5 % of hemicellulose solubilisation for sorghum straw when biomass was treated for 60 min in an autoclave at 121 ºC and 0.75 % NaOH. Other studies have shown the effectiveness of pretreating lignocellulosic materials with dilute alkali for xylan removal by soaking soybean or wheat straw biomass at room temperature [29, 30]. However, these results are difficult to compare with the present work due to very different process conditions for alkali treatment. In the integrated one step process of the present work, the action of alkali is combined with the mixing and shearing effects of extrusion during the 2 minutes the residence time of the material inside the extruder at selected screw speed. Also working on extrusion, Jacquemin et al. [31] have reported effective hemicellulose extraction by a combined twin-screw alkali- extrusion in a mixture of wheat bran and straw, but in this work much higher values of R [close to 50% (w/w)] are employed.

According to our results, R value ≥ 5% during extrusion is needed to provoke significant hemicellulose solubilization, regardless extrusion temperature. On the other hand, when the results of lignin content variation during extrusion are analyzed, AIL content is below that "concentrated" value (18.2%) at R of 5 and 7.5% at all temperatures tested, while only at the lower value of 2.5 %, no lignin was removed. This means that mildest conditions are required to attain some lignin solubilization compared to hemicellulose, suggesting greater effectiveness of alkaline extrusion treatment on lignin component, in the conditions tested in the present work.

A positive effect of alkali treatment on lignin hydrolysis and swelling of wheat straw biomass has been reported in relation to an improvement in the enzymatic digestibility of the substrate [32]. In fact, it is generally recognized that lignin is one of the main hindrances that difficult the enzyme access to the cellulose [33] and for this reason, delignification is often used as an indicator of the pretreatment effectiveness in alkaline pretreatments.

As a consequence of hemicellulose and lignin solubilization as R increases from 2.5% on, cellulose is concentrated in the extrudate, reaching maximum content close to 65 % w/w, which is obtained at 50ºC and R of 7.5%. The increase of the cellulose content in extrudate at this condition compared to untreated BS (39.1% dwb) is an important advantage of biomass pretreatment for ethanol production, since the material that is introduced in the following step of hydrolysis is cellulose-enriched in relation to the untreated material.

In order to assess the importance of the effect of T and R on the variation of extrudate composition, a response surface analysis was performed with the experimental data shown in Table 3. The graphs reveal a marked effect of R and, in a much lesser extent, of T, on cellulose, hemicellulose and lignin content, as it is shown in Figures 1, 2 and 3 (panel A), respectively. There is a clear tendency towards increasing cellulose content and decreasing hemicellulose and lignin content in extrudate, as the value of R levels up from 5 to 7.5%. In fact, when the significance of each effect on the global behaviour of the variable is analyzed by the Pareto charts (panel B of Figures 1-3); only the effect of R is significant, at a 95.0% confidence level. The effect of temperature is not statistically significant for any of the considered responses. It means that in the range of variation of temperature tested no significant influence on extrudate composition exists, given the effect of NaOH/BS DM ratio.

Table 3 also includes values of solid content of extrudates at different conditions, which varies between about 14 and 30% dwb. In the integrated extrusion process of this work, it depends mainly on the different inlet flows and the extrusion configuration, since no treatment is given to the raw material before. This implies that the consistency (solid content) of the extrudate at the output can be adjusted according to the process needs by varying the flows and the screw profile, always considering the effect on solids losses, which can be attributed to any carbohydrate solubilisation.

Enzymatic hydrolysis

From glucan and xylan enzymatic hydrolysis yields values on extrudates produced at different extrusion conditions shown in Table 3, it can be concluded that integrated NaOH-extrusion improved enzymatic digestibility, in comparison to untreated BS (17 and 10% of theoretical for glucan and xylan, respectively, data not shown). The hydrolysis yield refers to the efficiency of enzymes to reach and hydrolyze the carbohydrates in the extrudate and is often referred as "saccharification efficiency". It levels up as R increases to maximum values of 90% and 88% of theoretical for glucan and xylan conversion, respectively. This means that the enzymatic digestibility of glucan and xylan in BS can be increased in 5 and 9 times, respectively, in relation to untreated BS by the moderate integrated alkaline-extrusion process of the present work.

The increase of R causes a marked effect on enzymatic digestibility, regardless the temperature tested. At the lowest R value of 2.5%, where no significant changes in extrudate composition in relation to raw BS were found, the rise in EHY compared to untreated BS is significantly lower than obtained when R increases to 5% on, as a result of a higher destructuration of the material at a more severe extrusion conditions. When BS is extruded at 5% R, a marked increase in EH yield is found at all temperatures, attaining the maximum value of 90% in the experiments at 100ºC. At this alkaline condition, lignin solubilization was found (see discussion above), which can be considered an indicator of a break of the strong cellulose–hemicellulose–lignin association resulting in enhanced enzyme accessibility. However, in experiments at 7.5% R, the increase of T to 75 and 100ºC, results in a decrease in enzymatic digestibility. The combination of more severe alkali and temperature conditions may be leading to condensation and/or repolymerization reactions in the cellulose-hemicellulose matrix, resulting in lower enzymatic hydrolysis efficiency.

On the other hand, xylan conversion yield increases in all temperatures tested as alkaline conditions become more severe, attaining values of 88% of theoretical in extrudates at 75 and 100ºC and 7.5% R. It means that the digestibility of xylan is greatly improved by the one-step alkaline extrusion process, which shows the positive effect of this pre-treatment to deconstruct lignocellulose matrix and facilitate the action of hemicellulolytic enzymes. As in glucan hydrolysis, the improvement with T is statistically significant, which is well supported by the response surface graphs shown in Figures 4 and 5 that show the marked effect of alkali ratio in both responses EH of glucan and xylan. The Pareto charts confirm that only the effect of R is significant at a 95.0% confidence level.

A positive effect of extrusion in enzymatic digestibility of lignocellulosic substrates has also been demonstrated in other lignocellulosic substrates. De Vrije et al. [7] studied a combination of mechanical and chemical pre-treatment methods for the production of fermentable sugars from *Miscanthus* and concluded that a combination of extrusion and sodium hydroxide is more favourable based on higher cellulose conversion yields and good performance of the pre-treatment. More recently, Yoo et al. [9] have reported on the increase of glucan to glucose conversion yield of soybean hulls from 40.8% of untreated biomass to 94.8% after extrusion at 80ºC and 350 rpm in a twin-screw extruder, without any catalyst addition. However, in the comparison with our results it is necessary to consider that soybean hull has much lower lignin content (2.3%) than barley straw, which makes the digestibility of this feedstock quite high even when untreated. Other authors report on sugar recovery yields after enzymatic hydrolysis (sugar released during saccharification related to sugar in raw material) being positively affected by an extrusion process alone [2, 5, 6], in combination with chemicals [4, 12, 19, 34, 35] or with other pretreatment techniques, such as liquid hot water [10]. Alkali impregnation and subsequent extrusion of prairie cord grass was studied by Karunanithy and Muthukumarappan [4] in the optimum conditions of 114ºC extrusion temperature, 122 rpm screw speed, 1.70% alkali concentration, and 8 mm particle size. They obtained a maximum glucose, xylose and combined sugar recoveries of 86.8, 84.5, and 82%, respectively, by enzymatic hydrolysis.

Regarding the mechanism underlying the positive effect of extrusion in enzymatic hydrolysis efficiency, it is not completely elucidated, although several hypotheses have been formulated. According to Yoo et al. [9], extrusion causes disruption of cell wall structure due to the combination of thermal and mechanical energy. This combined effect would lead to exposure of greater surface area and also to deconstruction of hemicellulose chains, which interfere in enzymes accessibility [19]. In the integrated alkaline-extrusion process of the present work, the extrusion effect would be enhanced by the action of the alkaline agent, which promotes glucan conversion by degradation of ester bonds and cleavage of glycosidic linkages in the cell wall matrix leading to the reduction of the lignin-hemicellulose complex and swelling of cellulose [14]. Our results support the fact that there is a positive effect of combining extrusion and alkali treatment (in the interval of T: 50-75ºC and R: 5 -7.5%) on enzymatic digestibility of extruded BS. It occurs at the same time that a part of hemicellulose and/or lignin solubilises, indicating substrate destructuration. However, when T is increased to 100ºC, enzymatic digestibility decreases.

Other approaches using a combination of techniques that includes alkali have also been shown to be effective to enhance enzymatic digestibility of barley straw. Persson et al. [25] pretreated barley straw by impregnation with different amounts of NaOH, followed by steam explosion at 190 ºC and varying times. They found glucose conversions after 72h between 80 and 90 % and xylose yields ranging from 83 to 95 %., which are in the range of those obtained in the present work: However, the integrated alkaline-extrusion poses the advantage of an one-step integrated process at lower temperature and lack of sugar degradation compounds. As in the present work, the authors observed a tendency towards higher glucan conversion with higher NaOH concentration in the impregnation step.

Optimization of extrusion conditions for enzymatic hydrolysis of glucan and validation of the model

Experimental data of enzymatic hydrolysis of glucan were processed using Statgraphics in order to infer a mathematical model which would describe the system and be able to predict its behaviour, being the final objective to maximize the conversion of glucan to glucose. This analysis was characterized by regression parameter R^2=0.937, which indicates that the model as fitted explains 93.7% of variability for glucan hydrolysis yield. The adjusted R^2 statistic was 0.833. The program gave the following equation: $X= 20.36-1.57467\ R+ 0.74393\ 3T+0.600\ 8R^2-0.02168\ RT-0.004088T^2$ for EH yield of glucan (X), as a function of NaOH/DM ratio (R) and temperature (T).The contour plot depicted in Figure 4 shows how the design leads us towards an optimal experimental condition within the experimental range studied, which was found to be 89.9% of theoretical, at R= 6% and T=68ºC.

To test the model validity, a new experiment was carried out, setting the process variables to the optimum ones given above. EH yield for glucan at optimum conditions was 88.9% of theoretical, which is in agreement with the predicted value (89.9% according to the above formula) and confirms the validity of the model. At the optimum conditions, the composition of extrudate gave the following composition: cellulose, 54.9%, hemicellulose, 28.9% and lignin, 14.7% (dry weight basis). The hydrolysis yield for xylan resulted in 71% of theoretical and the amount of hemicellulose-derived sugars reached 10.3 g/l. The absence of furfural and HMF in filtrate was confirmed in this validation experiment.

Conclusions

The results obtained in this work prove the effectiveness of the integrated one-step alkaline extrusion technique to enhance enzymatic digestibility of barley straw biomass for sugar production. Values of saccharification efficiency of glucan and xylan in extruded BS can be increased in 5 and 9 times, respectively, in relation to untreated material in EH experiments on washed extrudate at 5% (w/w) solids load. These results confirm the effectiveness of the integrated pretreatment to cause destructuration of lignocellulose structure, so promoting the accessibility of enzymes to the carbohydrates during hydrolysis step.

The study of the influence of process variables, such as temperature (T) and NaOH/barley straw DM ratio (w/w) (R) showed that only R has a significant effect on enzymatic hydrolysis yield and extrudate composition variation, in the interval studied of 2.5-7.5% (w/w) and 50-100ºC. The optimization of process variables for a maximum glucan to glucose conversion by enzymatic hydrolysis led to optimal conditions of R=6% and T=68ºC. At these conditions, glucan yield reached 89.9% of theoretical, while xylan conversion was 71 %. These results support the great potential of this one-step combined pre-treatment technology for sugar production from lignocellulosic substrates. The absence of sugar degradation products is a relevant advantage over other traditional methods for a biomass to ethanol production process since inhibitory effect of such products on sugar fermentation is prevented. Further efforts will be devoted to continue studying the process conditions that result in a more integrated and effective process and to test its feasibility at a larger scale.

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