SUGAR PRODUCTION FROM BARLEY STRAW BIOMASS PRETREATED BY COMBINED ALKALI AND ENZYMATIC EXTRUSION

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Abstract

A pretreatment that combines a thermo-mechanical process (extrusion) with chemical and biological catalysts to produce fermentable sugars from barley straw (BS) biomass was investigated. BS was firstly extruded with alkali and then, the pretreated material (extrudate) was submitted to extrusion with hydrolytic enzymes (bioextrusion). The bioextrudate was found to have 35% (w/w dwb) of total solids in soluble form, partly coming from carbohydrate hydrolysis during bioextrusion. About 48% of soluble solids dry weight is comprised by sugars, mostly glucose and xylose. Further enzymatic hydrolysis of bioextrudate could be successfully carried out at high solid loading level of 30% (w/v), with sugar production yield of 32 g glucose and 18 g xylose/100 g bioextrudate at 72h incubation (equivalent to 96 and 52 g/l concentration, respectively). These results, together with the high level of integration of the process, indicate a great potential of this pretreatment technology for sugar production from lignocellulosic substrates.

Keywords: lignocellulose, biomass, extrusion, enzymatic hydrolysis, alkaline treatment

1 Introduction

Lignocellulosic biomass is the most promising feedstock to be used in biorefineries, as source for the production of biofuels, chemicals and other biomass-derived products (Uhilein and Schebek, 2009). This type of biomass has a heterogeneous composition, which includes

cellulose, hemicellulose, starch, lignin, oils and proteins. Specific technologies need to be developed to achieve a complete fractionation of the material and the conversion of each fraction to high-value products (Menon and Rao, 2012).

Lignocellulosic biomass pretreatment technologies have been widely investigated over the last years as a first and crucial stage to breakdown fibre structure and make carbohydrates accessible to hydrolytic chemical or biological catalysts. Various biological, chemical, and physical pretreatment approaches have been proven to open the recalcitrant structure of lignocellulosic biomass and increase the susceptibility of cellulose to enzymatic attack. (Mosier et al., 2005; Tomás-Pejó et al., 2011). However, most of these pretreatments achieve these goals by solubilizing hemicelluloses and/or lignin that in turn can be degraded to compounds that can exert inhibitory effect on enzymes and microorganisms in the subsequent steps, due to the use of elevated temperatures and/or high concentration of chemicals such as solvents, acids, etc. On the contrary, twin-screw extrusion stands out as a technology that can effectively open the recalcitrant structure of lignocellulosic biomass into its constituents at mild temperature and chemicals conditions, preventing the formation of such inhibitory by-products (Duque et al., 2013). Other advantages are that it can be operated in continuous way and that produces little effluent, disposal and solid losses.

Extrusion process is based on the effect exerted by the tight rotation of a single or a twin-screw inside a stationary barrel equipped with temperature control. The most common equipment for the extrusion of biomass is the fully intermeshing co-rotating twin-screw extruder, whose configuration can be varied by the combination of different types of screw elements, which produce different effects, such as transport, mixing and shearing, along the extrusion process (Sentruk-Ozer et al., 2011).

The application of extrusion in biomass pretreatment has been lately investigated as individual technique (Karunanithy and Muthukumarappan, 2010a, 2010b, 2011a, 2011c; Karunanithy et al., 2012; Yoo et al. 2011; Zhang et al., 2012), or in combination with chemicals, such as alkali, ammonia or ethylene glycol, in the so called “reactive extrusion” (Dale et al., 1999; Karunanithy and Muthukumarappan, 2011b, 2011d, 2013; Lamsal et al., 2010; Eckard et al.,

2011; Lee et al., 2010). Although the process has been successfully demonstrated without catalyst on a variety of substrates such as switch grass, prairie cord grass and corn stover (Karunanithy and Muthukumarappan, 2010a, 2010b, 2011a), the integration of extrusion and chemical agents may result in more efficient biomass component fractionation. In the particular case of alkaline-extrusion, several approaches have been carried out by soaking the biomass with an alkaline solution prior to its introduction in the extruder. For instance, Karunanithy and Muthukumarappan (2011d) optimized the reactive extrusion conditions for switchgrass reaching a maximum sugar recovery after enzymatic hydrolysis of 86.8 for glucose and 84.5 % for xylose, after soaking biomass for 30 min in 2% (w/v) alkali solution prior to extrusion. Zhang et al. (2012) also demonstrated the effectiveness of this sequential alkali-soaking/extrusion pretretament in enhancing sugar release by subsequent enzymatic hydrolysis in corn stover. Significantly, a step towards the integration of the alkali and extrusion pretreatment was taken in a previous work carried out by the authors (Duque et al., 2013), by introducing the alkaline solution directly into the extruder and running the whole process in a continuous way. In that work, NaOH and water were fed into the extruder and after extrusion, extrudate was collected, washed and used as substrate in enzymatic hydrolysis tests. The yields of enzymatic hydrolysis of the washed extruded material in the best conditions (6% NaOH/DM ratio and 68ºC) were 88.9% for glucose and 71.3% for xylose (values in % of the maximum theoretically achievable if all glucan and xylan contained in extrudate were transformed to sugars), in experiments at 5% solids and 27 FPU/g glucan enzyme load. However, it was necessary to extensively wash the extruded material before the subsequent step of enzymatic hydrolysis to remove catalyst and neutralize for enzymatic hydrolysis that implied high water consumption. Recently, Um et al. (2013) have also reported on the effect of chemicals, such as alkali, in the extrusion of rapeseed straw in a continuous twin-screw reactor, by feeding directly the catalyst into the extruder at a similar NaOH/DM ratio, but at higher temperature of 170ºC. The positive effect of alkali addition is shown by a 2.4 fold increase in enzymatic digestibility in comparison to untreated material, reaching a maximum of 60% in experiments at 2% solids loading. The authors claim an important role of particle size reduction during extrusion in the effectiveness of the process,

based on significant improvement of enzymatic digestibility also in experiments conducted by feeding hot water instead of alkali.

The first objective of the present work is to develop an advanced integrated alkaline extrusion process that includes a neutralization step into the extruder. This advanced process configuration allows avoiding the washing step of extrudate and thus, the water consumption at this stage. Moreover, this integrated process allows generating a pretreated material at high solid loading level, which can be used directly in a subsequent step for sugar production. The alkaline-extrusion process of the present work aims at enhancing the action of the alkaline agent, which promotes glucan conversion by cleavage of H_2 bonds of cellulosic structure and as a consequence, swelling of cellulose (Carrillo et al., 2005). Moreover, the work addresses an additional goal by performing a second extrusion run on the alkaline extruded biomass with hydrolytic enzymes to provide a good interaction enzyme-substrate at the high solids conditions in which process is carried out. Thus, a "bioextrudate" is produced, which can be directly incubated for sugar release from carbohydrates by enzyme action, without any additional downstream operation. The composition and characteristics of extruded materials and the sugar production of bioextrudate in subsequent enzymatic hydrolysis incubation at high solid loading is investigated.

2 Material and Methods

2.1 Raw material

Barley straw (BS) (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.

2.2 Twin-screw extrusion pretreatment

2.2.1 Twin-screw extruder

Extrusion experiments were carried out in a twin-screw extruder (Clextral Processing Platform Evolum® 25 A110, Clextral, France), composed of 6 modular barrels of 100 mm length. In each barrel, different screws of 25 mm diameter are fitted to provide transport, mixing and shearing effects. A computer into the extruder controls the extruder barrel temperature and the screw speed. A volumetric feeder KMV KT20 (Ktron) connected to the extruder is used to provide continuous biomass feeding.

2.2.2 Alkaline pretreatment

The operational parameters for alkaline extrusion of BS were selected based on results from a previous optimization study described elsewhere (Duque et al., 2013). Accordingly, extrusion of BS was carried out at an even temperature of 68ºC and at a ratio of NaOH/BS dry weight (w/w) of 7.5%. The screw profile used in this step is shown in Figure 1A. Alkaline pretreatment was run by continuously feeding BS biomass through the feeder at a feeding rate of 0.6 kg/h fresh weight and a NaOH solution (10% w/v) through a metering pump connected to the extruder in module # 2. The present work integrates a neutralization step by feeding a solution of diluted phosphoric acid (1% w/v) in module #4. The aim is to get the extrudate at the output with a pH value from 5-5.5. Both NaOH and phosphoric acid solution flows were adjusted to provide a liquid flow to solid input ratio (L/S) inside the extruder of 12/1. This value had been previously shown to provide a proper operation during this stage.

Regarding screw profile (Fig 1A), in the first two barrels conveying elements were installed to transport the biomass forward, while in the third barrel a mixing section was included to achieve a good mixture between the biomass and the catalyst. Two reverse screws, one in module #4 and the other immediately after module #5, were set to increment the pressure and shearing effect in the extruder. A filtration unit was set up in module #5 to separate a liquid fraction or filtrate before extruded material output. The residence time of the biomass in contact with the soda can be estimated in less than 1 min, considering the total time of the extrusion run (about 2 min) and the sequence of the different steps (material feeding, alkaline treatment, neutralization and filtration) during the run.

After extrusion, a portion of the extrudate was dried, milled and analysed for major components as described below. Likewise, the filtrate was collected and analysed for sugars and degradation compounds, i.e. furfural and HMF, following the methodology shown below.

2.2.3 Extrusion with enzymes (bioextrusion)

The extrudate produced in the alkaline extrusion was used as feedstock in a second run for extrusion with hydrolytic enzymes (bioextrusion). Extrudate, with moisture content around 63%, was continuously fed into the extruder through the feeder at a flow of 0.62 kg/h fresh weight. The screw configuration in this step is shown in Figure 1B, aimed at providing transport and mixing effects with no shearing effect of reverse screws. Extrusion run was performed at an even temperature of 50ºC and no filtration was applied in this step.

Some exploratory trials at different L/S ratios using water instead of enzymatic solution carried out to optimize this value indicated that L/S ratio close to 4/1 could provide a proper operation within the extruder. The amount of enzyme added into the extruder was 25 mg protein/g dry extrudate, which can be considered in the range of those used in other research works by the authors about enzymatic hydrolysis of steam explosion-pretreated BS (García-Aparicio et al., 2011). The enzyme solution fed into the extruder was prepared by mixing cellulase and hemicellulase preparations, in a ratio 9:1 in protein content, in citrate buffer 200 mM, pH 4.8. Novozymes A/S (Denmark) kindly provided the enzymes. The dilution of enzymatic preparation and its flow-feeding rate into the extruder was calculated considering the ratio enzyme/dry matter into the extruder of 25 mg protein/g dry extrudate and the selected L/S ratio of 4/1.

After bioextrusion experiments, a portion of the bioextrudate was dried at 40ºC, milled and analysed for major components as described below for raw and extruded biomass characterization. Moreover, a fraction was submitted to a characterization process as follows.

2.2.4 Characterization of bioextrudate

To examine if hydrolysis of carbohydrates occurs during bioextrusion due to enzyme action, a portion of bioextrudate was filtered and divided into two fractions: insoluble (IF) and soluble (SF). IF was washed and dried at 40ºC to determine main components content following the procedures shown below for raw and extruded biomass. The liquid resulting from washing the bioextrudate was analysed for simple sugars by HPLC and the amount of sugars measured considered being a part of the SF.

The volume of SF was recorded and a portion centrifuged at 9000 rpm. An aliquot of the supernatant was analysed by HPLC as described below for sugar content. In parallel, an aliquot was submitted to mild acid hydrolysis (4% (v/v) H_2SO_4, 120ºC and 30 min) and analysed again for total sugar content. By difference between the former (monomeric sugars) and second (monomeric plus oligomeric sugars) values, the amount of oligomeric sugars was calculated. The rest of the supernatant was dried until constant weight to determine total dissolved solids (TDS) content.

2.3 Sugar production of bioextrudate upon incubation at high solid loading level

To evaluate the sugar production efficiency of extruded material after bioextrusion, the bioextrudate was incubated in a Terrafors-IS bioreactor 15 l capacity (Infors HT, Switzerland) for 72 hours. Solid content of bioextrudate was 23% w/w, which is equivalent to a 30% solids level (w/v) in the incubation media. To follow-up sugar release during incubation, samples were withdrawn at 0, 3, 6, 8, 20, 24, 48, 72 h, boiled to inactive enzymes and measured for glucose and xylose concentration by HPLC as described below in analytical methods section.

To determine if any sugar is found in the media sample in oligomeric form, in parallel an aliquot of the sample was submitted to mild acid hydrolysis as described above (see point 2.2.4), and the amount of glucose and xylose oligomers calculated.

The efficiency of whole sugar production by enzymatic hydrolysis taking into account the bioextrusion and further incubation of the bioextrudate (hereinafter sugar production yield) was calculated. Glucose and xylose (as monomers and oligomers) were measured in the incubation media and divided by the potential glucose/xylose that could be found based on glucan/xylan

content in the bioextrudate (dwb). Results are expressed in percentage. For this calculation, the values of glucose and xylose found in the media at 0 time were considered since these sugars were produced during bioextrusion prior to incubation.

2.4 Raw and extruded biomass chemical composition analysis

National Renewable Energy Laboratory (NREL, CO) laboratory analytical procedures (LAP) for biomass analysis (NREL, 2007) were used to determine carbohydrates, acid-insoluble lignin and ash content in raw and extruded materials (extrudate and bioextrudate).

Granulometric analysis was carried out on untreated BS and extruded biomass samples according to European Standard Method [European Standard Norm, 2010]. The aim is to analyse differences in particle size distribution before and after alkaline and enzymatic extrusion.

2.5 Analytical methods

The filtrate recovered after alkaline extrusion and the soluble fraction of the bioextrudate were analysed for their content in monomeric and oligomeric sugars (glucose, xylose, arabinose, galactose). The oligosaccharides ratio was determined as the difference in monomeric sugar concentration before and after mild acid hydrolysis. Simple 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. (2007). Likewise, sugars concentration after completion of enzymatic hydrolysis tests were measured in EH media by HPLC as described above. Furfural and HMF were analysed 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).

3 Results and Discussion

3.1 Raw Material and extrudate characterization

The significance of dry matter composition in barley straw (Table 1, first column) has already been discussed in a previous work by the authors (Duque et al., 2013). Based on its high total carbohydrate content of 65% (dwb) (39.1 % cellulose and 25.7 % hemicellulose), this feedstock has been proposed as a very promising substrate for sugar production and conversion to high added-value products such as bioethanol.

Regarding extrudate composition (Table 1, second column), the material is somehow enriched in cellulose, xylan, and acid-insoluble lignin in relation to untreated BS due to the solubilisation into the filtrate of easily removable biomass components such as extractives, soluble ash (Duque et al., 2013) and a minor part of glucose and hemicellulosic-derived sugars, mostly xylose (see below, liquid fraction analysis). The amount of solids in the extrudate, (37%) are 95% insoluble solids and 5% soluble since the material is not washed after extrusion and a minor fraction of soluble solids remain in the extrudate after the filtration step carried out into the extruder. These soluble solids contain negligible amounts of monomeric sugars as glucose and xylose.

Extrudate is made up of 73% of carbohydrates, 17% lignin and 4.5% ash. The fact that the extruded material contains virtually all glucan and xylan from raw material, at the conditions tested in this work, stands out for a clear difference with other pretreatment techniques using water or chemicals that cause extensive solubilisation of easily removable carbohydrates (Alvira et al., 2010; Ballesteros et al., 2011; Mosier et al., 2005). It may represent an advantage from the point of view of increased potential sugars to be released by enzymatic hydrolysis, if the material becomes accessible to enzymatic attack. To demonstrate this point is one of the objectives of this work.

Extrusion has been reported to cause physical disruption of cell wall assembly due to the combination of thermal and mechanical energy (shearing forces), leading to a certain deconstruction of biomass structure (Yo et al., 2011). Moreover, the addition of alkali during extrusion may cause hemicellulose and lignin extraction as well as cellulose cristallinity

decrease, depending on operating conditions. As a result, non-structural components are easily released, the lignin-hemicellulose complex is somehow affected and swelling of cellulose occurs (Eckard et al., 2011). This swelling effect can be observed in the extrudate generated in this work in comparison to untreated BS. The extrudate appearance is remarkably different from untreated BS by presenting a more soft and porous aspect. In addition, granulometric analysis shows changes in particle size distribution between both materials (Figure 2). About 63% of the particles in the raw barley straw are over 3.15 mm, while this percentage decreases to 44% in the extrudate. Instead, the fraction between 3.15 and 0.52 mm particle size increases in the extrudate and sums up to 53.7%. These data are in agreement with the observations of Um et al. (2013) about the reduction of particle size due to the friction and shearing forces inside the extruder.

3.2 Filtrate composition

The characterization of liquid fraction or filtrate from alkaline extrusion is shown in Table 2. The pH value close to 5 indicates successful neutralization in the extruder, which is important to facilitate its use directly in the second extrusion run with enzymes. Regarding sugars content, the values found are low, in all cases < 2g/l. Data represent sugars in both monomeric and oligomeric form; in the case of glucose, around 40% is found as monomer, while for xylose this figure is only 3%. Concentration values are highly dependant on the total liquid flow and so, values in relation to the amount of BS loaded have been calculated. The value of glucose concentration found in the filtrate is equivalent to 2.4% of the glucose contained in raw material, which is solubilized during extrusion. The amount of xylose solubilized (1.5% of BS DM) represents close to 6% of the content in raw material.

Alkaline treatments have been shown to remove hemicellulose and lignin from lignocellulosic biomass, but its effectiveness is closely related to the concentration and conditions of alkali treatment. The relative mild alkaline conditions tested in the present work do not result in significant hemicellulose removal, although the enzymatic accessibility of the extruded material is significantly increased in comparison to raw BS (Duque et al., 2013), due to a swelling effect

of alkali on the cellulosic fibres. On the contrary, Jacquemin et al. (2012) have reported effective hemicellulose extraction by a combined twin-screw alkali- extrusion in a mixture of wheat bran and straw, but employing much higher values of NaOH/DM [close to 50% (w/w)]. It is important to highlight that neither furfural nor hydroxymethyl furfural (HMF) were detected in the filtrate, which is a clear clue of no sugar degradation during this step of the pretreatment process.

3.3 Bioextrudate characterization

The analysis of bioextrudate composition shown in Table 3 allows estimating the mass balance of solids between the two fractions: soluble and insoluble. Whole bioextrudate solid recovery (108%) is over 100% due to enzymes and buffer that are added during bioextrusion, representing a factor of 1.08 in relation to solids extrudate dry weight. Therefore, carbohydrates and lignin content of bioextrudate (in percentage dwb of bioextrudate) is slightly lower than in extrudate (Table 1), due the above "dilution" factor.

Total solids of the bioextrudate are composed of 35% soluble and 65% insoluble solids. Almost a half of the soluble solids (48%) are comprised by sugars, mostly glucose and xylose, but also minor amounts of galactose and arabinose. A great part of the xylose found in SF is in form of oligomers (60%), while glucose oligomers only represent a 12% of the total glucose in soluble form. The fraction of glucose in SF (8.7%of whole bioextrudate dwb) accounts for 18% of total glucose content of whole bioextrudate (47.4% dwb). Likewise, xylose in SF (6.8% of whole bioextrudate dwb) represents a value close to a 28% of total xylose (24.5% dwb).Thus, it is demonstrated that by the addition of enzymes into the extruder in the second run, the feedstock for this stage (extrudate) is transformed into a bioextrudate that contains a part of the carbohydrates already in soluble form. This is the key difference with extrudate and the evidence that enzymes are acting during bioextrusion.

It is relevant to highlight that while during bioextrusion some carbohydrate breakdown occurs, no carbohydrate losses take place and the rest of the carbohydrates are available for further hydrolysis upon further incubation of the material.

Regarding differences in appearance between bioextrudate and extrudate, the former presents dark brownish colour, is sticky, and tent to lump, which may be related to changes in lignin chemistry and solubilisation of carbohydrates. Results of particle size distribution by granulometric analysis in both materials (Figure 2) show that particle size is reduced in bioextrudate. There is a negligible amount of particles over 3.14 mm, while the fraction of particles between 3.14 and 0.52 mm reaches 60% of the total. Particles under 0.52 mm account for 39%, compared to just 2.3 % of the particles under that size in extrudate. These results prove that bioextrusion results in a material with distinctive physical characteristics in relation to the extrudate.

3.4. Sugar production yield on bioextrudate

Bioextrudate was incubated in the 15 l bioreactor at 50ºC during 72h to determine sugar production yield. Values of production yield at 0, 3, 6, 8, 20, 24, 48 and 72h, as well as concentration in g/l (both monomers and oligomers) are shown in Figure 3, for glucose and xylose (panels A and B, respectively).

As commented in point 3.3., the enzymes action produce a certain carbohydrate hydrolysis during the second run in the extruder, which confirms that saccharification is taking place to a certain extent during bioextrusion. As a result, there is an initial amount of sugars in the media at 0h, that accounts for 7.5% of the potential glucose and 18% of the potential xylose. These values are a bit lower than the amount of soluble sugars found when characterising the bioextrudate due to the different methodologies used for their quantification (see Materials and methods). During the first 20 - 24h of incubation, the hydrolysis of glucan and xylan is slow and the values are low, but then, within the next 48h, it accelerates until reaching production yields of 73% and 72% of theoretical for glucose and xylose, respectively. Glucose (monomers and oligomers) concentration in EH media at this condition after 72 hours incubation reached 96 g/l. The delay for the hydrolysis to take off can be due to the high solids content at which the experiment is carried out, 30% w/v. It is indeed well known that increasing the solids loading presents some difficulties such as mass transfer limitations and end product inhibition of

enzymes by glucose and cellobiose, which may affect the final efficiency of hydrolysis (Wang et al., 2011; Lu et al., 2010). However, the fact that in the present work the enzymes are thoroughly mixed during the bioextrusion and that some sugars are released by the enzymatic action during this step, seems to provide an advantage when the material is further incubated at extended time, resulting in reasonably good yields at 30% w/v solids. Moreover, the use of advanced enzymatic cocktails consisting in cellulase boosted with xylanase is with certainty influencing this positive result. Supporting this idea, Manzanares et al. (2012) have reported a positive effect of xylanase addition on SSF of steam-exploded forage sorghum that allows increasing solid concentration up to 18 % (w/w) with good SSF performance and high final ethanol content in SSF broth.

The event of xylan hydrolysis during incubation with a reasonably good efficiency, results in a significant amount of xylose being released to the media, which would be available for conversion to valuable products in a further step. At 30% solid loading level, the final concentration of xylose is 52 g/l (considering monomers and oligomers). A certain amount of other free sugars, mostly arabinose (up to 6 g/l) and galactose (3 g/l) are also found by HPLC (data not shown), resulting in a total concentration of fermentable C5 and C6 sugars about 155 g/l, from which 71 g/l are monomeric glucose and 31 g/l correspond to monomeric xylose. The values of sugar production referred to dry matter of extrudate reach values about 32 g glucose/100 g dry extrudate and 18 g xylose/100 g extrudate. Considering the production of glucose and xylose in relation to both glucan and xylan in the extrudate, a *combined* sugar production yield of 73% can be calculated. These results are somewhat lower than those obtained in other lignocellulosic biomasses submitted to alkaline extrusion, such as prairie cord grass (Karunanithy and Muthukumarappan, 2011b) or switch grass (Karunanithy and Muthukumarappan, 2011d). For the first one, the optimal extrusion conditions of 114ºC, 122 rpm and soaking in 1.7% alkali, yielded a maximum combined sugar recovery of 82%. In the work on switch grass biomass, maximum combined sugar recovery of 88% was recorded after extrusion of the material at 180ºC and 118-rpm screw speed, previously soaked for 30 minutes in an alkali solution of 2% (w/v) concentration. However, it is important to highlight that these

experiments were obtained in enzymatic hydrolysis experiments at low solid loading level at less than 5% solids, so resulting in low sugar concentrations in the media. The fact that the combined extrusion process of the present work results in reasonably high yields at high consistencies up to 30% (w/v), stands up for a clear advantage in terms of sugar concentration in hydrolysis media. Moreover, the high level of integration of the steps in the whole process represents an important gain over other technologies that require separate processing steps.

In brief, the process described in the present work denotes several important benefits over other extrusion processes and pretreatment techniques described in the literature for biomass fractionation and enzyme accessibility improvement. On the one hand, the steps of alkaline treatment and neutralization are integrated in a single run, which provides the possibility to use directly the material in the second run without downstream operations. On the other hand, the solid loading level of the pretreated extrudate can be adjusted, so that a high solids material can be produced, which is a clear advantage against other pretreatment processes that use steam or other catalysts. Finally, the introduction of the enzymes in a second extrusion run provides an enhanced enzyme-substrate mixture, which results in a pretreated material with a fraction of carbohydrates already solubilized due to enzyme action, without carbohydrates losses. As a result, enzymatic hydrolysis at high solid loading level can be successfully performed, resulting in EH media containing high total sugar concentration up to 155 g/l available for fermentation to ethanol or other uses.

4. Conclusions

The results show the feasibility and effectiveness of the integrated combined alkali and enzymatic extrusion process to pretreat barley straw biomass for sugar production. After two sequential runs of alkaline plus enzymatic extrusion (bioextrusion), saccharification has already started in the produced bioextrudate. The bioextrudate can be incubated at high solid loading for sugar release, giving reasonably high sugar productions yields and concentration in EH media. These results support the great potential of this integrated process as pretreatment technology.

Further studies should be carried out to test new enzymatic mixtures and evaluate its feasibility at a larger scale.

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