Study of process configuration and catalyst concentration in integrated alkaline extrusion of barley straw for bioethanol production

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Abstract

Barley straw was pretreated with alkali (NaOH) at increasing NaOH/DM ratios (6 to 10%) and neutralized in a twin-screw extruder, obtaining a substrate ready for the incubation with enzymes. Two process configurations were evaluated: with and without filtration inside the extruder. The enzymatic digestibility of the pretreated substrates was evaluated and filtration was proved to be beneficial for the enzymatic hydrolysis. A maximum enzymatic hydrolysis yield of 71% was attained for the barley straw pretreated with 8 g NaOH/100g dry barley straw and filtration inside the extruder. The ethanol production was evaluated on this substrate for increasing solid loadings, 2.5, 15 and 20% (w/v). A global process yield based on ethanol production, including hydrolysis and fermentation, was calculated over the pretreated material in experiments at 20% (w/v) solids, reaching 53% of the maximum theoretical. The concentration of ethanol reached up to 29 g/l in these conditions.

Keywords: lignocellulose; biomass; extrusion; alkaline treatment; enzymatic hydrolysis; ethanol production

1. Introduction

Lignocellulosic biomass is increasingly being recognized as an important feedstock for the production of biofuels and chemicals by contributing to environmental sustainability and not competing with food production [1]. Particularly, bioethanol produced from lignocellulose by biochemical pathway (*cellulosic ethanol*) is foreseen to become one of the most credible alternatives to meet bioethanol supply targets in the next years in a sustainable way [2]. A great effort has been devoted in the past decades to overcome the technological and process-based barriers present in the different stages of the cellulosic ethanol value chain, i.e, pretreatment, hydrolysis, fermentation and distillation. However, the highly-structured and recalcitrant nature of lignocellulose remains as an important bottleneck in the conversion process and thus any advances in the pretreatment step will undoubtedly contribute to optimize hydrolysis and further processing downstream operations [3]. Several pretreatment approaches based on elevated temperatures and/or high concentration of chemicals such as solvents, acids, etc. have been proven to effectively fractionate lignocellulosic biomass and extensive literature has been published on this subject in past years [4, 5, 6, 7, 8]. Recently, thermo-mechanical extrusion process has been claimed as an interesting and alternative method for fractionation of lignocellulosic biomass based on it can be applied at relatively mild process conditions and operates in continuous way [9]. It provides high shear, rapid heat transfer, effective and rapid mixing, and a continuous throughput and adaptability for many different process configurations [10, 11, 12]. One consequence of its versatility is that extrusion can be used alone or combined with other pretreatments or chemicals (reactive extrusion) to enhance its performance.

Concerning reactive extrusion with alkali, two different forms of combining alkaline pretreatment with extrusion have been described in the literature on different biomasses. One is the impregnation of the raw biomass in an alkali solution for a determined period of time, followed by the introduction of this wet biomass into the extruder [10, 13, 14, 15, 16]. Another way is the addition of the alkaline solution directly into the extruder [17, 18, 19, 20], which allows one-step process operation. The second alternative is more advantageous, considering the

reduction in the process time and the higher integration level of this strategy. Particularly for barley straw biomass, the authors proved in a previous work [17] that twin-screw extrusion in combination with alkali directly fed into the extruder effectively fractionates barley straw (BS) biomass into its constituents. Mild temperature and chemicals conditions were used, preventing the formation of inhibitory byproducts coming from the degradation of hemicellulose/lignin. However, it was also shown that the so produced materials (hereinafter, extrudates) need a washing or pH-adjusting step before they can be submitted to enzymatic hydrolysis (EH). Therefore, downstream operations become complicated, meaning a significant drawback from an industrial point of view.

The process strategy followed in the present work is the alkaline extrusion of barley straw by performing continuous alkaline-extrusion at reasonably low NaOH/DM (w/w) ratios (R) ranging from 6 to 10% (w/w), with neutralization inside the extruder. These R values were selected considering the effective results obtained in the former work of alkaline extrusion of BS [17].

The fact that neutralization is performed during extrusion run increases the integration of the pretreatment and allows generating a substrate ready for the enzymatic hydrolysis [21]. However, in this integrated concept the addition of both alkali and acid inside the extruder implies the formation of inorganic salts that remain in the substrate for its subsequent hydrolysis and/or fermentation. Thus, in this work the inclusion of a filtration step inside the extruder was studied aimed at evaluating if a partial removal of such soluble solids affects the yield of carbohydrate hydrolysis upon further incubation of the extrudate with cellulolytic enzymes. The strategy including filtration is compared to one without filtration. The effect of increasing NaOH/DM ratio in the integrated alkaline pretreatment of BS and the inclusion of the filtration step in the performance of enzymatic hydrolysis of extrudate was evaluated in laboratory tests using commercial enzymes.

Moreover, this paper deals with one of the less studied areas in the assessment of the extrusion pretreatment: the evaluation of the ethanol production potential from alkaline extrudates [9]. For this purpose, simultaneous saccharification and fermentation (SSF) experiments at increasing solids loading levels, including a 24h liquefaction step, were carried out on extrudates generated on the best process conditions. The production of ethanol was measured and the yield of the fermentation process calculated.

2. Materials and Methods

2.1 Raw material

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

2.2 Alkaline extrusion pretreatment

Extrusion experiments were carried out in a twin-screw extruder (Clextral Processing Platform Evolum® 25 A110, Clextral, France) described elsewhere [17]. The extruder was continuously fed with 0.6 kg/h of BS. A solution of NaOH (10% w/v) was pumped into the extruder at variable feeding flows, in order to achieve alkaline ratios of 6, 8 and 10 g NaOH/100 g BS. After alkaline treatment and in the same extrusion run, a solution of H_3PO_4 (0.1M) was introduced for neutralization purposes, in a proportion enough to obtain a pH about 5 at the output, which is an adequate value for the hydrolytic enzymes action in the subsequent step comprised in this work. Temperature was set at 68ºC along the extruder and the speed of the screw was 150 rpm, as in the previous work [17], which means a residence time inside the extruder of about 2 min.

Two process configurations were considered in order to carry out the alkaline pretreatment and neutralization of the biomass: the complete configuration (C) (Figure 1), in which alkali and

acid are sequentially added to the extruder and then extrudate comes out, and the filtered configuration (F) (Figure 2), which includes a filtration step previous to the output of the extrudate. The position of the inputs and barrels, as well as the screw profile, were adapted and optimized to each of the configurations. In C, alkali is introduced in barrel 1 and acid in barrel 5, while in F alkali is also introduced in barrel 1, but acid is added in barrel 4 and barrel #5 is replaced by a filtering module. Transport screws, bi-lobal paddles and reverse screws were used in order to provide transport, mixing and shearing effects along the extrusion.

An important factor, influencing the effectiveness of the pretreatment is the liquid to solid ratio inside the extruder (L/S) [9]. This parameter is calculated as the sum of the liquid flows (NaOH solution for the reaction zone, soda plus acid solution flow in the neutralization zone) entering the extruder, plus the moisture of the raw material, divided by the amount of dry weight barley straw fed to the equipment. It is especially important in the reaction zone (barrels 1 to 4 in C and 1 to 3 in F). The values for the L/S in the reaction and in the neutralization zone for the three soda levels are shown in Table 1.

To stabilize operation conditions, the extruder was operated for 30 minutes before any sampling was taken. After this time, the extrudates in configuration C and F and the filtrate from configuration F, were collected for at least 30 minutes to avoid variation in output flow rates. Extrudates were kept in sealed plastic bags and stored at 4ºC until use. A portion of extrudates and filtrates were analyzed for main components and the content in monomeric and oligomeric sugars and potential inhibitors (furfural, hydroxy-methyl furfural – HMF, and phenols), respectively, as described below.

2.3 Raw and pretreated material characterization

The composition in carbohydrates, acid-insoluble lignin and ash content was determined in raw material and extrudates, according to the laboratory analytical procedures (LAP) for biomass analysis [22] of the National Renewable Energy Laboratory (NREL, CO).

2.4 Enzymatic hydrolysis tests

Extrudates from both configurations (C and F) were submitted to enzymatic hydrolysis in 0.05 M sodium citrate buffer (pH 4.8) at 50ºC and 2.5% (w/v) dry extrudate load. 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 (protein content basis) was added in a dosage of 20 mg protein (8 FPU of cellulase)/g dry extrudate. The enzymes were kindly provided by Novozymes A/S (Denmark). Samples were taken at 0h and then at 24h and 48h, when the hydrolysis of the extrudates is completed. After withdrawal, samples were boiled for 10 min to deactivate enzymes before chromatographic analysis. The sample at 0h is used to determine the amount of sugars coming from the solubilisation during extrusion and from the enzymatic preparation. These sugars do not result from the hydrolytic action of enzymes during incubation and therefore, they are substracted from the final amount of sugars when calculating the actual yield of the hydrolysis in the course of incubation.

The parameter used to assess differences in the enzymatic hydrolysis performance by incubation of the extrudates produced in the two process configurations and increasing R values is the enzymatic hydrolysis yield (EHY). This parameter is calculated 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 of theoretical. As it was previously explained, the sugars solubilized at t = 0h are substracted in this calculation. In addition, the overall production yield in the extrudate was also calculated, taking into account sugars produced during both the extrusion step and the additional incubation. So the amount of sugars at the beginning of the hydrolysis is summed up to that resulting from incubation to calculate this yield, which is expressed as g of sugar (glucose and xylose) produced per g of dry extrudate. In all calculation, the amount of glucose coming from the enzyme preparation was substracted.

2.5 Ethanol production from extrudates

Extrudates were submitted to simultaneous saccharification and fermentation (SSF) tests with a previous 24 h step of liquefaction (L) at 50ºC (hereinafter, LSSF). Experiments were carried out at 2.5, 5, 10, 15 and 20% (w/v) solids loading in Erlenmeyer flasks incubated in rotary shakers at 150 rpm as explained in point 2.4.

To start the experiment, 20 mg protein/g of extrudate of the above mentioned enzymatic cocktail (see point 2.4) were supplied to each flask and they were incubated for 24h at 50ºC. After that, hydrolyzates had liquefied and the flasks were inoculated with 1g/l of pre-grown *S. cerevisiae* (see below) and further incubated at 35ºC for another 72h. Samples were withdrawn every 24h. Glucose and ethanol concentration were measured as described below in section 2.6.

Saccharomyces cerevisiae (Ethanol Red from Fermentis, France) was prepared for inoculation by growing 0.5 g of the dehydrated microorganism on a rotary shaker at 150 rpm and 35ºC for 16h, in growth medium composed by: 30 g/l of glucose, 4 g/l of yeast extract, 2 g/l of $(NH_4)SO_4$, 1 g/l of KH_2PO_4 and 0.3 g/l of $MgSO_4 \cdot 7H_2O$. The preculture was centrifuged at 9000 rpm for 10 min at 4ºC and the supernatant was discarded. The pellet was resuspended in citrate buffer (0.05mM) in a volume calculated to achieve inoculum load of 1 g/l.

To evaluate ethanol production on extrudates, LSSF yield was calculated considering both steps, liquefaction and subsequent SSF. Thus, the yield was calculated as the amount of ethanol produced during SSF, divided by the potential production of ethanol in hydrolyzates and expressed in %. Potential ethanol was determined as the maximum ethanol that could be produced from the glucose in extrudates, considering 100% hydrolysis yield and the theoretical conversion factor 0.51 g ethanol/g glucose.

2.6 Analytical methods

Sugars content in the filtrate and in EH media after completion of enzymatic hydrolysis tests was measured by high-performance liquid chromatography (HPLC) in a Waters 2695 liquid chromatograph with refractive index detector, as described in Cara et al. [23]. The

oligosaccharides ratio in the filtrate was determined as the difference in monomeric sugar concentration before and after mild acid hydrolysis (3% v/v H2SO4, 121 ºC and 30 min).

Furfural and HMF and phenolics (i.e., hydroxy-benzoic acid, hydroxy-benzaldehide, vanillic acid, vanillinand cumaric and ferulic acids), 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 H2SO4 and 11% acetonitrile at a flow rate of 0.7 mL min-1. Column eluent was detected with a 1040A Photodiode-Array detector (Agilent, Waldbronn, Germany).

Ethanol concentration was measured by gas chromatography using an Agilent technology 7890A GC System equipped with an Agilent 7683B Series injector, a flame ionization detector (both maintained at 150ºC) and a column of Carbowax 20 M at 85ºC.

3. Results and discussion

3.1. Characterization of raw material, extrudates and filtrates

Six pretreated extrudates were obtained from extrusion experiments, corresponding to the three levels of alkali (6%, 8% and 10% g NaOH/g BS) in both configurations, C and F. The composition of the raw BS and extrudates is shown in Table 2.

It can be seen that complete extrudates (CE) have a similar, or even a bit lower, content of hydrocarbons compared to the untreated BS (36 vs. 39% of cellulose, 24 vs. close to 26% hemicellulose). This is due to the fact that the extrudate is not filtered and, therefore, all the components remain in the substrate for analysis. It must be noticed, however, that there is a small dilution factor resulting from the addition of chemicals (alkali and acid) during the pretreatment, which sums up an additional weight in the complete extrudate and could be accounted for the slight differences found between CE and raw BS composition. The higher

amount of ash in the complete extrudates (from 12.8% ash at 6% R to 14% ash at 10% R) in comparison to raw BS supports this fact.

On the other hand, filtered extrudates (FE) show a higher proportion of cellulose and hemicellulose than in untreated BS, about 41-45% and up to 28.5%, vs. 39.1 and 25.7%, respectively. In this configuration, part of the inorganic salts and other non-structural compounds is removed by filtration, which results in concentration of major components to a certain extent. The analysis of the filtrate (Table 3) shows minor concentration of sugars (expressed as simple glucose and xylose after mild acid hydrolysis) coming from carbohydrate solubilisation. The amount of solubilized glucose is small, ranging from 1 to 2 g/l. Considering the volume of filtrate recovered during filtration, which is different in each trial, these concentration values are equivalent to 1.9 - 2.8% of the glucose in the BS, with no significant variation among R values. These small amounts of glucose solubilized could be attributed to the presence of some starch in the raw material, coming from grain contamination. However, the solubilisation of xylose is more significant, up to 5 g/l; and increases significantly with R between 6-8% R to 10% R. These differences account for 6.5 to 17.3% of the xylose in the raw BS, again depending on the volume of filtrate. It is important to note that more than 96% of the solubilized xylose was originally found in oligomeric form in the filtrate, whereas oligomers of glucose were about 64%. The solubilisation of combined C6 and C5 sugars related to their content in raw BS varies between 4 and 8.5%. In spite of solubilizing this small amount of sugars, filtration concentrates the carbohydrates in FE to some extent (as it was mentioned above) due to extensive solubilisation of minor components such as extractives and ash.

Regarding inhibitory compounds (Table 3), neither furfural nor HMF were found in the filtrates. This is an expected result found in extrusion experiments at low temperatures and one of the main advantages of this pretreatment over other thermo-chemical methods that are carried out at higher temperature conditions. Phenols were found in negligible concentrations (< 0.1 g/l, which means less than 0.01% of dry BS), while acetic acid was the main compound detected with a concentration about 4 g/l. This value, referred to the volume of filtrate and to BS dry

matter, means that 95% of the acetyl groups in the raw BS (about 1.8% dwb, data not shown) have been removed from the hemicellulose by alkaline extrusion. This deacetylation effect is in agreement with the results described in the literature and it is thought to occur by saponification of acetyl ester bonds [24, 25]. The amount of acetic acid that is removed with the filtrate is, however, still present in CE, which could interfere with the enzymatic activity. However, the extent of this effect is highly related to the concentration value, as it was proved in a study by Hodge et al. [26], who demonstrated inhibition effect but at much higher acetic acid concentrations of 15 g/l.

3.2. Enzymatic hydrolysis efficiency

The values of EH yields at 24 and 48h, depicted in Figure 3A and 3B (for cellulose and xylan, respectively), show that glucose and xylose are mostly released within the first 24h of hydrolysis, which is consistent with low solids loading (2.5% w/v) and the enzyme dose used in the experiments.

The results from the statistical analysis show that there are not significant differences between the enzymatic hydrolysis yields of glucan of complete extrudates produced at different R levels ($p=0.05$), either in filtered ones. In spite of the NaOH level not being significant, the FE at 8% was the substrate which gave a slightly better glucan yield, i.e. 71% of theoretical. This means an improvement 4 times over the EH yield of glucan of untreated barley straw (17% of theoretical, data not shown). The yields from the hydrolysis of xylan were lower, varying in the range of 52 to 60% of the theoretical, which is a xylan EH yield up to 5.8 times higher compared to the untreated biomass (10% of theoretical, data not shown). In the case of xylan, there are statistically significant differences between CE and FE at 6 and 8% R, while this difference is not found at 10% R. The fact that the extrudate contains virtually all xylan from raw material and that it is hydrolyzed during incubation with a reasonably good efficiency results in a significant amount of xylose being released to the media. Xylose would be available

for conversion to valuable products in a further step, increasing the amount of total sugars produced in a single step of enzymatic hydrolysis.

The values obtained in the present study are somehow lower than those attained in a previous work from the authors [17] on alkaline-extrusion of barley straw. The extrudates produced were washed and submitted to enzymatic hydrolysis, resulting in a highest glucan yield close to 90% at 68ºC, 150 rpm and 6%R. The decrease found in the present work can be attributed on the one hand to changes in the configuration of extrusion profile, since the introduction of neutralization inside the extruder implies a shortening of alkaline treatment. On the other hand, it is significant that the extrudates are not washed after extrusion.

Um et al. [18] proved the greater effectiveness of NaOH-extrusion versus extrusion with hot water to pretreat rape straw, and achieved a maximum enzymatic digestibility value of 60% in the case of alkaline extrusion. This result was obtained at 1.5%R, 19 rpm and 170ºC, which means a lower catalyst consumption compared to the present study, but also the use of much more thermal energy due to the high temperatures used. Liu et al. [27] also employed a twin-screw extruder and NaOH loadings between 4 and 10% to pretreat corn stover, followed by a thermal preservation step. They found glucan and xylan conversions up to 83% for glucan and 89% for xylan. The fact that they use a high extrusion temperature (100ºC), a harsher screw profile and that they wash extrudates before EH, differentiates this study from the present work. The results Liu et al. [27] obtained were more similar to the ones in Duque et al. [21]. The fact of integrating a neutralization step in the extruder seems to shield the effect of the alkali concentration in the extrudates. The yields obtained in the present work are, nevertheless, quite acceptable and integrated neutralization bring other advantages from the operational and industrial point of view, which makes these configurations an interesting subject of study.

Lamsal et al. [15] washed extensively alkali-extruded wheat bran and soybean hulls previous to enzymatic saccharification, obtaining remarkable improvements in sugar recovery. The authors hypothesized that the washing step removed chemical treatment residues, as well as enzyme

inhibitors from the extrudates. Moreover, Zhang et al. [16] compared the glucose yields of washed and not washed extrudates pretreated with different alkali concentrations and came to a similar conclusion: washing of extrudates is beneficial at high alkali concentrations, although at lower concentrations the effect is the opposite.

The combination of alkaline treatment and extrusion inside the extruder has also been addressed by Kang et al. [19] on *Miscanthus* biomass and Han et al. [20] on barley straw. Both authors based the analysis of the effectiveness of the pretreatment on the biomass to ethanol ratio (BTER, a measure of the ethanol produced referred to glucose in raw biomass) and their results will be discussed later on in point 3.3.

Regarding the two different configurations, there are significant differences for a confidence level of 5%. In general, enzymatic digestibility of FE is higher than that of CE, proving a positive effect of soluble compounds removal on enzymatic hydrolysis performance. Thus, the hydrolysis of complete substrates would be somehow affected by some compounds present in CE, which are removed by filtration. The analysis of filtrates (Table 3) can shed light on the possible causes of the inhibition. Hodge et al. [26] stated that the inhibition of enzymatic hydrolysis at low solids concentrations is completely due to the soluble compounds. The sugars and acetic acid removed with the filtrates are still present at the beginning of EH in CE. As it was previously commented, most of the sugars were found in oligomeric form, mainly xylooligomers, and according Qing et al. [28] these oligomers have a remarkable inhibitory effect on cellulases. It is possible also, that a combined effect of soluble sugars and acetic acid is hindering the hydrolysis efficiency [26].

Another parameter to evaluate the effectiveness of the pretreatment is the sugar production, which will eventually determine the potential for the production of bioethanol in relation to the weight of extruded material. This parameter, expressed as g of sugar (glucose or xylose) per g of dry extrudate, sums up the sugars produced by alkaline extrusion (i.e. the sugars measured at the beginning of the EH experiments) to the ones produced during the incubation with enzymes and

refers them to the weight of the extrudate. Glucose and xylose production of CE was about 23 and 13 g/100 g dry extrudate, respectively. The EH essays of FE resulted in higher sugar productions, especially at 8%R, where the productions were 34 g of glucose and 16 g of xylose per 100 g of dry extrudate, equivalent to 71% of the glucan yield and 58% of xylan yield.

To advance with the study of ethanol production, the best extrudate was selected according to the results discussed above, and this is FE at 8%R.

3.3. Ethanol production and LSSF yield

Ethanol production tests were carried out on extrudate at best condition (FE, 8%R) at 2.5, 5, 10, 15 and 20% w/v solid loadings. The assays were first incubated 24h at 50ºC with the cellulolytic cocktail and then inoculated and incubated for additional 72h at 35ºC (liquefaction plus SSF, LSSF).

The results shown in Figure 4 indicate that LSSF yields increase progressively with the incubation time, reaching values up to 73% of theoretical and 53% of theoretical at 72h of incubation at 2.5 and 20% solids loading, respectively. The observed decrease of the yield with the increasing solids content is influenced by the progress of the EH at high solids loading, as it could be observed that liquefaction was hindered in the flasks at 15 and, mainly, 20% solids loading. This effect on the EH at high solids loadings is well documented in the literature [29, 30]. It has been reported that increasing the solids loading presents some difficulties such as high viscosity, end product inhibition of enzymes by glucose and cellobiose and mass transfer limitations, especially at laboratory flask scale. However, due to the higher concentration of potential cellulose at high loadings, the ethanol concentration in the media increases from 5 g/l at low solids loading up to close to 30 g/l at 20% (Figure 5).

To date, there are not many studies on the fermentability of extrudates in the literature. One work that addresses this issue is the paper from Yoo et al. [31]. They extruded soybean hulls at different in-barrel moisture contents and screw speeds and carried out separated hydrolysis and fermentation tests, obtaining a maximum ethanol production of 15.4 g/l (at initial hydrolysis

consistency of 10% w/w). The temperature was kept under 100ºC and no significant concentrations of furfural or HMF were detected, similar to what happens in the present study. Our results compare very favorably with this work, since about twofold ethanol concentration (28.7 g/l) is obtained at double solid level (20%). The authors also discuss on the effect of acetic acid released to the hydrolyzate by extrusion and EH, which can inhibit ethanol production, depending on concentration and pH. One of the advantages of the FC presented in the present paper is the possibility to avoid this potential problem in the hydrolysis and fermentation steps, by removing the acetic acid with the filtrate.

Kim et al. [32] studied the potential for ethanol production by SSF of poplar sawdust extruded with sulphuric acid. Cellulase load was 30 FPU/g cellulose. The authors report a decrease of the ethanol yield at solid loadings over 6% (w/v) and used a fed-batch strategy to overcome this problem, reaching a final concentration of 11% solids and 40 g/l ethanol without losing efficiency (77.3% yield calculated over the raw material). In our case, we also observe a decrease of the LSSF yield at 15 and 20% solids (w/v); therefore, the fed-batch strategy could be a good solution to be explored in future works.

Kang et al. [19] also dealt with fermentation of extrudates, in this case, from alkali-extruded *Miscanthus* at 200ºC and 12.8% R. They carried out SSF experiments at 25% substrate load, that resulted in 67 g/l of ethanol (26.8 g/100 g extrudate) and ethanol conversion, BTER, of 88%. These values are higher than the obtained in the present work; however it must be noticed that Kang et al. [19] performed a more severe pretreatment, washed the substrates after alkaline-extrusion and added a higher enzyme dosage (30 FPU/g cellulose). They also proved that an increase of the enzyme dose increases the amount of ethanol produced. This strategy could be applied in future studies to achieve higher LSSF yields from alkali-extruded BS.

Specifically working with alkali-extruded BS (16%R), Han et al. [20] obtained 46 g/l of ethanol after SSF with 30 FPU/ g cellulose of enzyme at 20% substrate load, equivalent to 23 g/100 g extrudate. The BTER at this consistency was 77%. Han el al. also observed that higher enzyme

load led to higher ethanol yields and that this ethanol yield decreased at solid loadings over 20% (w/v), which is consistent with the conclusions of the previous works [19, 32].

In comparison to the reported values, the ethanol concentration and LSSF yield obtained in this work at 20% solids (28.7 g/l and 53.5% of theoretical, respectively) are good given the present operational conditions (mild temperature, no washing of extrudates, 20 FPU/g cellulose). Nevertheless, the authors consider the experiments done are a preliminary assessment of the hydrolyzability and fermentability of extrudates and these steps require further optimization to achieve ethanol concentrations in the fermentation media enough to make the whole process profitable at industrial level.

4. Conclusions

A neutralization step was successfully integrated with alkaline extrusion of barley straw, producing a pretreated material suitable for direct incubation with enzymes. Two extrusion configurations were compared and the advantages of the inclusion of a filtration step inside the extruder were demonstrated. However, the effect of increasing R values in the alkaline treatment was not significant at the levels tested. Extrudates gave good hydrolysis yields, being the best around 70% of theoretical at low solid concentration. The capacity for ethanol production from the best extrudates (FE, 8%R) was tested through LSSF experiments and 19.7 g/100g extrudate ethanol were obtained after 72h incubation at 2.5% solid concentration. Figure 6 shows the material balance of barley straw to ethanol at the optimal conditions of extrusion shown in this work, calculated based on results obtained at 15% w/w solids, a more relevant process condition. Under these conditions it is possible to obtain 110 g ethanol from 1 kg barley straw.

5. References

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