RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

RSC Advances

RSCPublishing

ARTICLE

Sugarcane bagasse delignification with potassium hydroxide for enhanced enzymatic hydrolysis

Cite this: DOI: 10.1039/x0xx00000x

S. M. Paixão,*[1] S. A. Ladeira,[2] T. P. Silva[1], B. F. Arez,[1] J. C. Roseiro,[1] M. L. L. Martins[2] and L. Alves*[1]

Received 00th May 2015
Accepted 00th May 2015

DOI: 10.1039/x0xx00000x

www.rsc.org/

The optimization of an alkaline pretreatment process for the delignification of sugarcane bagasse (SCB) to enhance a subsequent enzymatic hydrolysis was performed accordingly to the Doehlert uniform shell design. In this experimental design, the effect of two factors, potassium hydroxide (KOH) concentration and autoclaving time at 121 °C (1 atm), on cellulose, hemicellulose, or total polysaccharides, and lignin contents in SCB was evaluated. This response surface methodology revealed that KOH concentration was the factor that most influenced the chemical characteristics of treated SCB (SCB_t), being the optimal conditions for the highest delignification: KOH in a range of 5-10% (w/v) and an autoclaving time of 35 minutes, attaining up to an average of 97% total polysaccharides without inhibitors accumulation (furfural, 5-hydroxymethyl furfural) and ≤5% lignin. SCB_t samples from two pretreatment conditions (KOH 3.25% - 13 min; KOH 10% - 35 min) were selected, based on the greatest delignification (70-74%) and polysaccharides availability (95-97%) after pretreatment, and further hydrolysed for fermentable sugar production. High sugar yields were obtained from both the pretreated samples (866 to 880 mg sugar/g biomass, respectively) in contrast with the 129 mg sugar/g raw biomass obtained from untreated SCB. These results demonstrate the effectiveness of alkali pretreatments with KOH, both improving the overall digestibility of raw SCB polysaccharides from about 18% up to 91%. But, the harsh alkali treatment (KOH 10%) is the most effective if the highest glucose/xylose ratio in the final sugar-rich hydrolysate is the goal. Hence, the use sugar-rich hydrolysates obtained from SCB_t as carbon source for industrial purposes may provide a sustainable and economic solution for the production of bio-based added-value products, such as second generation (2G) bioethanol.

I. Introduction

Sugarcane (*Saccharum officinarum* L.) is a perennial grass that predominantly grows in the tropical and subtropical regions and constitutes the world's largest crop, being Brazil the largest producer.[1] The sugarcane bagasse (SCB), a fibrous residue of cane stalks left over after the crushing and extraction of the sugar from sugarcane, it is the main brazilian agroindustrial residue being produced 250-280 kg per ton of sugarcane processed. Nowadays, about 50% of the SCB is burned to generate power for the alcohol distilleries and sugar mills, and a smaller portion is used for animal feeding. However, a great amount from the total SCB produced is still discarded as an agricultural waste leading to an environmental problem.[2-4] Moreover, aligned with an increasing global demand for ethanol fuel there is the prospect of increased sugarcane production resulting in a greater amount of SCB available.[5,6]

SCB is a lignocellulosic biomass containing significant amount of carbohydrates (60-70%), mostly in the form of two polysaccharidic molecules, cellulose (33-45%) and hemicellulose (28-35%), and a polyphenolic macromolecule, lignin (20-30%).[2-4] Cellulose, the most abundant component, is a polymer consisting of long unbranched chains of D-glucose units linked by β(1→4)-glycosidic bonds. Cellulose has crystalline and amorphous regions, being the former the main reason for its recalcitrance to the hydrolytic process. The second predominant constituent is hemicellulose, an amorphous, complex, branched and heterogeneous polysaccharide network, based on pentoses, hexoses and sugar acids. Thus, hemicellulose has a variable composition according to its source, and, in SCB it is composed of heteroxylans, with mostly xylose. Hemicellulose matrix can be chemically hydrolysed more easily than cellulose.[2] Lignin is a three-dimensional amorphous phenolic polymer, which holds the hemicellulose and cellulose fibres. It has a complex structure formed by the polymerization of aromatic alcohols that are resistant to enzymatic attack and degradation, and thus its content and distribution is recognized as the most important factor that determine the recalcitrant cell wall hydrolysis.[7,8,9]

Hence, SCB is an abundant waste and a renewable source of polysaccharides that could be available in amounts sufficient to provide a source of sugars for biotechnological processes. Indeed, there is a great interest in the SCB exploitation as a potential low-cost feedstock for industrial purposes, since it may provide a sustainable and economic solution for the production of bio-based added-value products, such as 2G ethanol, xylitol, organic acids, enzymes, single-cell protein, etc.[3,5,10-12] However, to achieve significant yields of fermentable sugars from SCB, usually it is

necessary to apply prior pretreatments to the lignocellulosic biomass to turn it more amenable to further saccharification and fermentation, which involve costs. The pretreatment technologies applied to lignocellulosic substrates are required to reduce the recalcitrance, hydrolyse hemicellulose and lignin and consequently improve the yield of fermentable sugars that are released by enzymatic hydrolysis.[13-15] Pretreatments may decrease the crystallinity of cellulose and/or the degree of polymerization, increase the accessible surface or selectively remove the hemicellulose and lignin from the lignocellulosic matrix. An effective pretreatment technology should not only disrupt biomass for easy accessibility of cellulases and hemicellulases during enzymatic hydrolysis, but also minimize the degradation of fermentable sugars and avoid degradation products (inhibitors), as furfural and 5-hydroxymethyl furfural (HMF), which affect both enzymatic hydrolysis and microbial fermentation.[16-20]

Different pretreatment processes for lignocellulosic biomass, such as SCB, have been investigated including physical (grinding, irradiation), chemical (alkali, acids, solvents, supercritical fluids), physico-chemical (steam explosion) and biological (white-rot fungi, bacteria) approaches.[3,13,16,21,22] Among them, some alkali-based treatments have been proposed with considerable success. Dilute alkaline solutions lead to rupture of the lignocellulosic cell walls to dissolve the lignin, hemicellulose and silica, hydrolyzing uronic acids and esters of acetic acid and by swelling cellulose.[16,23,24] The decomposition of lignin is generally attributed to the cleavage of α-aryl ether linkages of polyphenolic compounds, while hemicellulose depolymerisation and solubilisation, to monosaccharides and oligosaccharides, and cellulose swelling are due to the weakening of the hydrogen bonds.[11]

Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline pretreatments,[25] but most studies on alkaline pretreatment of lignocellulosic biomass thus far have utilized NaOH, from 0.25-10% (w/v),[16,26,27] for the pretreatment process. It is described that sodium hydroxide, when applied as biomass pretreatment, promotes the greatest degradation and higher yields in the subsequent fermentation processes in comparison to other alkalis, such as sodium carbonate, ammonium hydroxide, and calcium hydroxide, and to hydrogen peroxide.[12,16,28] However, recently Ong et al.[29] and Sharma et al.[30] reported the higher effectiveness of KOH treatment over NaOH treatment in their studies on pretreatment of rice straw and switchgrass, respectively, for fermentable sugar production. Potassium hydroxide is a relatively less explored pretreatment agent but may potentially be used for lignocellulose pretreatment due to its reported reactivity with carbon nanofibers and carbon nanostructures[31] and its ability to deacetylate biomass.[32,33] Low cellulose crystallinity and lignin content are key features towards good sugar yield during enzymatic hydrolysis of pretreated biomass, but significant levels of deacetylation can also increase digestibility even at moderate lignin content and high crystallinity index. However, for a low lignin content, crystallinity index and acetyl content do not have a significant impact on enzyme digestibility.[29,30,32]

Alkali pretreatment for lignin removal from lignocellulosic biomass is still one of the drawbacks in industrial scale ethanol production because it substantially increases the overall production cost and also contributes to environmental issues.[34] Thus, there is a great need to develop cost-effective processes for delignification of lignocellulosic biomass aiming to get the highest fermentable sugar production on its subsequent hydrolysis, as well as the lesser toxic inhibitory byproducts as possible, towards further biotechnological application, such as bioethanol production. In this work, the potential of KOH as a viable alternative alkali agent for the SCB recalcitrant biomass delignification based on its different reactivity patterns compared to NaOH was explored. In this context, the main goal consisted on the optimization of the biomass KOH pretreatment towards the highest lignin removal and carbohydrates retention for enhanced subsequent enzymatic hydrolysis. Hence, the effects of KOH concentration and autoclaving time at 121 °C (1 atm) on the levels of cellulose, hemicellulose, or total polysaccharides, and lignin in SCB were evaluated according to an experimental design, following the Doehlert distribution for two factors.[35] The treated SCB samples with the greatest delignification or polysaccharides availability were selected and further hydrolysed using an enzymatic cocktail consisting of commercial enzymes mixture. The effectiveness of the delignification pretreatment by KOH was evaluated through enzymatic hydrolysis performance and reducing sugar yield estimation.

II. Materials and methods

A. Lignocellulosic biomass

The sugarcane bagasse (SCB) used in this work was kindly provided by the sugarcane plant "Paraiso" located in the city of Campos dos Goytacazes, Rio de Janeiro, Brazil. It was thoroughly rinsed with distilled water for removal of particulate materials and sugar residues, and dried on a dehydrator Pardal PE60 at approximately 60 °C for 48 hours. Then SCB was further grounded in a Willy mill, 35 mesh sieve, and stored under refrigeration until be used.

The chemical composition of this washed and further milled SCB was analysed according to the NREL (National Renewable Energy Laboratory, USA) analytical procedures.[36] The average composition of this feedstock was: 4.35% moisture, 42.43% cellulose, 28.96% hemicellulose, 18.61% insoluble lignin and 1.47% ash. This percentage is relative to g/100 g biomass.

B. Alkaline pretreatment with KOH

To remove the lignin from the milled SCB, an alkali pretreatment consisting on autoclaving this lignocellulosic biomass in different concentrations of KOH (%, w/v aqueous solutions) for different periods of time was performed. Specific amounts of SCB (10 g) were placed in 70 mL of KOH solutions (liquid (mL)/solid (g) ratio of 7) with different concentrations and then subjected to a heat treatment in an autoclave (121 °C) for different time periods. Each treated SCB (SCB_t) warm (40-50 °C) was vacuum filtered through a fritted buchner filter funnel, rinsed with warm distilled water until neutral pH and then oven dried at 60 °C for 24 h before stored in plastic containers at room temperature until used. All solids were quantified and considered in solid recovery calculations for each treatment. The effects of KOH concentration and time of autoclaving at 121 °C on the level of cellulose, hemicellulose, lignin and total polysaccharides in SCB were studied according to a statistical experimental design.

C. Methodology of the experimental design

Experimental distribution for two factors, according to the Doehlert uniform shell design[35] was used to produce response surfaces. Fourteen experiments (7 conditions + 7 duplicates) were carried out within an experimental domain, with KOH concentrations ($X1$) between 1% and 10% and the time of autoclaving at 121 °C ($X2$) between 10 and 60 minutes. The coded representation of the variables was used for calculation purposes. The responses studied (Yi) in this design were: levels of cellulose, hemicellulose, lignin and total polysaccharides in SCB. The model used to express each response was a second-order polynomial model: $Yi = \beta 0 + \beta 1X1 +$

$\beta 2X2 + \beta 12X12 + \beta 11X1^2 + \beta 22X2^2$, where *Yi* corresponds to the experimental responses *i*, β are parameters of the polynomial model and *X* is the experimental factor level.

D. Analytical methods

D.1. Chemical characterization of treated SCB – About 2 g of each SCB_t was analysed according to the NREL analytical procedures[36] to determine their composition in total polysaccharides (cellulose and hemicellulose), lignin, ash, acetic acid, furfural and 5-hydroxymethylfurfural (HMF). The moisture content was measured using the oven-drying method (105 °C to constant weight). Through a quantitative acid hydrolysis of the samples, with 72% (w/w) H_2SO_4 (60 min, 30 °C) followed by hydrolysis with 4% (w/w) H_2SO_4 (60 min, 121 °C), their content in terms of glucan, xylan, arabinan, and acetyl groups was analysed. The remaining insoluble contents were then ignited in a muffle-furnace, at 540 °C for 4 h, for ashes determination. The acid insoluble residue of each sample was considered as Klason lignin, after correction for ash. The percentages of cellulose, hemicellulose, lignin and ash were calculated on a dry weight basis and are the mean value of duplicate determinations.

D.2. Chemical characterization of hydrolysates - The acidic and enzymatic hydrolysates, with pH ≤5, were analysed by high performance liquid chromatography (HPLC) for the determination of the sugar content (glucose, cellobiose, xylose, arabinose) aiming the estimation of the cellulose and hemicellulose levels, and for the presence of some inhibitors (furfural, hydroxymethylfurfural) and/or organic acids (acetic acid), using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). All samples were filtered through 0.45 µm membranes, and pH adjusted whenever necessary, before analysis.

E. Enzymatic hydrolysis

Enzymatic saccharification assays were performed for the selected SCB samples, with a solid loading of 5% (w/v) in 5 mL total volume (50 mM sodium citrate buffer, pH 5.0 containing the enzymatic cocktail). The enzymatic cocktail applied consisted of a commercial enzymes mixture: Accellerase® 1500 (cellulase enzyme complex; 250 µL g^{-1} biomass), Accellerase® XY (xylanase enzyme complex; 50 µL g^{-1} biomass) and Accellerase® BG (beta-glucosidase enzyme complex; 90 µL g^{-1} biomass), which were kindly provided by Genencor, DuPont Industrial Biosciences (Palo Alto, CA, USA). The saccharification mixtures were incubated in an orbital shaker, at 50 °C and 150 rpm for 72 h. Controls (bagasse without enzyme cocktail; enzyme cocktail in buffer without biomass) were incubated at the same conditions. After 72 h, the hydrolysates were centrifuged (10,000 rpm for 20 min) and the supernatants were analysed by HPLC, as above described, to quantify the amount of sugars released (g L^{-1} of glucose, cellobiose or xylose) and the hydrolysis yields were calculated accordingly Maurelli et al.[37] equation:

$$\text{Hydrolysis yield (\%)} = \frac{X-H}{X-SCB} \times 100$$

considering X-H as the amount of sugar (X) detected in the hydrolysate (H), where X is glucose, cellobiose or xylose, and X-SCB as the amount of sugar in the dry weight SCB sample (pretreated ou untreated) before saccharification. Sugar values were quantified in duplicate for the saccharification assays.

F. **Scanning electron microscopy**

Samples of SCB, untreated and treated with KOH as described above, previously oven-dried (moisture less than 10%) were set manually on conductive double-sided tape and placed in a aluminium "stub" for observation in the scanning electron microscope, Hitachi, model TM 3000 (Hitachi, Tokyo, Japan) using an electron beam of 15 kV. Electron micrographs were taken at 150x and 500x magnifications.

III. Results and discussion

A. Levels of polysaccharides and lignin in SCB treated

Alkali pretreatment can be performed from room temperature to high temperatures (21-121 ºC), and times ranging from minutes to days.[25-27, 30] In this work, the coupling of autoclave (121 ºC/1 atm = 15 psi = 0.1 MPa)[26] and alkali pretreatment was chosen as a faster approach towards lignin removal from SCB (residence times of 10 - 60 min.). Steam explosion (SE), a hydrothermal treatment that is commonly used as pretreatment of biomass for hemicellulose separation, is also based in both physical and chemical methods to break the structure of the lignocellulosic material. In fact, SE promotes the defibration and the autohydrolysis of the lignocellulosic biomass with hemicellulose removal, which significantly improve the further substrate digestibility.[13,38-40] In this context, the KOH pretreatment in autoclave can permit to achieve a faster SCB deconstruction through lignin matrix solubilization and, indeed, enhance its further enzymatic hydrolysis and consequently the subsequent bioconversion of the fermentable sugars.

Thus, preliminary studies to delignify SCB were carried out through thermal alkali pretreatments of the biomass, at 121 ºC and 1 atm for 30 min, using two strong bases (NaOH and KOH) at 10% (w/v).[27,41,42] The ratio of biomass to liquid (alkaline solution) tested was 1:7, corresponding to 14.3% w/v of solids loading. The KOH pretreatment permitted a reduction of SCB lignin content from 19% to 5% with a cellulose content increase from 42% to 80%, while the NaOH pretreatment achieved a treated biomass with 7% of lignin and 72% of cellulose. In addition, the hemicellulose content in treated SCB was reduced from 29% to 14% and 11%, using KOH and NaOH respectively, and a high solid loss (%) was observed in both pretreatments (54% for KOH *vs* 56% for NaOH). Thus, these results pointed out for the pretreatment with KOH as the most effective towards lignin removal from SCB and simultaneously increasing the overall polysaccharides content (mainly cellulose). Despite KOH be more expensive than NaOH, its higher efficiency in treating this recalcitrant material makes it a promising tool. Moreover, the KOH may be recovered from the black liquor and further used as fertilizer. Therefore, the main goal of this work was the optimization of SCB delignification using KOH for enhanced enzymatic hydrolysis through the Doehlert uniform shell design.

This statistical approach evaluated the effect of KOH concentration (ranging from 1% to 10%) and autoclaving time at 121 °C (ranging from 10 min to 60 min) as factors that influence the effectiveness of the extraction of lignin from SCB. Table 1 shows the results of experiments following a surface response methodology in accordance with the Doehlert distribution for two factors. In this table, other results of the chemical characterization of SCB_t samples (ash, acetic acid, furfural, HMF) are also presented.

From the analysis of Table 1, it can be seen that when the time varies from 13.4 to 56.7 minutes maintaining the KOH at 7.75% (tests: 11, 12, 7 and 8), the overall profiles of the average levels of cellulose, hemicellulose, total polysaccharides and lignin in SCB_t did not show a relevant difference. In the same way, when a lower concentration of KOH was used, 3.25% (tests 9, 10, 13 and 14), the

changing of the autoclaving time caused similar responses. These observations show that the autoclaving time at 121 °C is not a key factor in the process. The extraction of lignin using KOH 3.25% or 7.75% was similar; however the levels of cellulose and hemicellulose varied despite the content of total polysaccharides have also been kept similar. By keeping a constant autoclaving time of 35 minutes, the increase of KOH concentration resulted in the increasing of the average levels of cellulose (47.4% to 68.9%) and total polysaccharides (75.5% to 95.4%) in the SCB_t (tests 1 to 6), however the average levels of hemicellulose decreased (from 28% to 16.6%). The extraction of lignin by autoclaving for 35 minutes was more effective when KOH 10% was used, yielding SCB with low levels of lignin (4.53-5.24%; tests 3 and 4). The extraction of lignin increases about 70% for a 10-fold variation in KOH concentration (1% to 10%), but the lignin removed with KOH 5.5% or 10% was very similar, reaching similar average levels of total polysaccharides. Only the ratio cellulose/hemicellulose varies with the increase of KOH concentration of 5.5% to 10% (tests 1 to 4). According to Table 1, the highest rates of delignification were obtained in the treatments with KOH concentrations ≥ 3.25%. Despite the high loss of solid biomass that occurred during these pretreatments (>41%), a high total polysaccharides content is available in all corresponding SCB_t samples.

The results obtained from the experimental design, namely the levels of cellulose, hemicellulose, total polysaccharides and lignin, were then fitted to a polynomial model in a regression analysis, to estimate how both factors influenced the responses.

Table 2 shows the different parameters derived from the model: β0, which represents the analysed response in the centre of the experimental domain; β1 and β2, which indicate the influence of the individual factors (KOH concentration and autoclaving time, respectively) on responses; β12, which indicates how both factors interact with each other and the influence on the responses; and β11 and β22, which determine how the response surface folds downward (negative values) or upward (positive values) quadratically, with a higher or lower slope depending on the magnitude of the absolute value.[43]

For all responses studied, the value of β1 is much larger than the value β2, meaning that the concentration of KOH virtually controls the treatment process (Table 2). The level of interaction (β12) is also small but it is larger than the contribution of the autoclaving time towards the final responses.

The values of the β parameters in the models expressing the levels of hemicellulose and lignin display negative values. β1 for hemicellulose is a larger negative parameter than the value of the β1 negative parameter for lignin. The effect of these values on both responses is observed in Table 1. Maintaining a constant autoclaving time of 13.4 minutes and changing the concentration of KOH from 3.25% to 7.75% the level of hemicellulose in the SCB_t drops 32% (tests 9, 10, 11 and 12). The same range of KOH tested for an autoclaving time of 56.7 minutes produced the same 32% decrease in the level of hemicellulose (tests 7, 8, 13 and 14). At the same conditions, the increase of KOH concentration had no effect on the level of lignin.

Figures 1 and 2 present the variation of the cellulose and total polysaccharides within the experimental domain. Both responses increase with the concentration of KOH and the vertical shape of the plots in the response surfaces show the limited weight of the factor "autoclaving time" on responses, being the pictorial representation of the relative values of β1 and β2 (Table 2). In Fig. 2, it can be observed that the pretreatment using KOH in concentrations between 5% and 9%, independently of autoclaving time, allows obtaining a high level of total polysaccharides (>96%). At high concentrations of KOH (>9%) the total polysaccharides increase due to the raise of cellulose (Fig. 1), counterbalancing the decrease in hemicellulose (Fig. 3).

Figures 3 and 4 show the variation of the levels of hemicelluloses and lignin within the experimental domain. These plots also represent the effect of the negative value of the β parameters, i.e. the values of the responses decrease as the KOH concentration increases. The autoclaving with higher KOH concentrations produced lower levels of hemicellulose (Fig. 3) and lignin (Fig. 4), but the cellulose level increased in the SCB_t (Fig. 1). Figure 4 indicates that the best treatment conditions to apply to SCB for lignin extraction are: autoclaving time less than 35 minutes and KOH concentration between 5% and 10%. These conditions allow a reduction of lignin in SCB from 18.6% to levels around 5%, with an increase of total polysaccharides to levels greater than 95%.

Additionally, to further clarify the actual importance of using autoclave (or a reactor with pressure control) over an incubator, at 121 ºC, for the SCB biomass delignification process, a control assay using an incubator was also carried out for the pretreatment condition in which the highest polysaccharides content was achieved (KOH 10%, 35 min at 121 ºC). The SCB_t obtained in this assay presented about 90% of total polysaccharides (71% of cellulose, 19% of hemicellulose) but still about 10% of lignin, which translates only 47% of delignification yield. These results seem to point out for the importance of pressure factor towards a more effective lignin matrix solubilisation during the SCB alkaline pretreatment followed by a greater increase of the total polysaccharides content (+ 7%). In fact, the treatment in autoclave (121 ºC and 1 atm) have permitted to achieve a biomass comprising up to ~97% of total polysaccharides and less than 5% of lignin, from a raw biomass containing 71% of total polysaccharides and 19% of lignin, demonstrating its overall effectiveness over the treatment using an incubator towards the highest SCB delignification (74% of lignin removal).

The reduction of lignin content in SCB allows greater accessibility to total polysaccharides (cellulose and hemicellulose), which can be an advantage when it is intended to apply enzymes (cellulases and xylanases) to generate sugar-rich hydrolysates for further 2G bioethanol or other added-value bioproduct production, using SHF (separate hydrolysis and fermentation) or SSF (simultaneous saccharification and fermentation) approaches.

B. Analysis of the experimental data

A statistical validation was performed to test the adequacy of the models to the sets of data. In this context, two tests were used: (i) F-test for the effectiveness of the factors; and (ii) F-test for the lack of fit. The former test is used to confirm if the source of variance, included in the residuals, results from an inadequacy of the models to reproduce experimental data; while the latter test is performed to detect if the origin of the variance was a result experimental error.[44] The results for both tests are presented in Table 2 including the significance levels evaluated for each F-test. The F-test for the effectiveness of the factors showed that a significant amount of variance in the data is explained by the factors, as presented in the models, meaning that the factors studied influenced the response obtained. This is demonstrated by the significance level of 1% ($\alpha = 0.01$), at which the null hypothesis (H0) can be rejected.

The F-ratio for the lack of fit for all responses is highly significant, since the null hypothesis can be rejected with a significance level ≤0.01. This shows that the lack of perfect prediction of the models can be explained by the experimental error, confirming the adequacy of the models for all sets of data presented.

Finally, Table 2 also shows the analysis of the model by the coefficient of multiple determination (R^2), indicating that only a

limited amount of the sum of squares corrected for the mean is accounted for by the residuals.

C. Enzymatic hydrolysis

SCB_t samples from two pretreatment conditions (KOH 3.35%, ~13 min *vs* KOH 10%, 35 min), both inducing high delignification in SCB (from 19% to ~5%), were selected for subsequent enzymatic hydrolysis with an enzyme cocktail (Accellerase® 1500, Accellerase® XY and Accellerase® BG) for fermentable sugar generation, in comparison to raw biomass (untreated SCB). Thus, the samples hydrolysed were respectively: untreated SCB; SCB_t with the highest cellulosic content (~80%) - replicates #3 and #4 (Table 1), and SCB_t with the highest hemicellulosic content (~31%) - replicates #9 and #10 (see Table 1).

The hydrolysis yields (percent cellulose and hemicellulose conversion) obtained after 72 h of saccharification of the three SCB samples tested are presented in Fig. 5. The results for enzymatic hydrolysis efficiency show that both cellulose and hemicellulose conversion was highly enhanced by both alkaline pretreatments applied to SCB in this work. Using pretreated biomass a conversion of cellulose of about 92% (SCB_t #3, #4) to 94% (SCB_t #9, #10), as well as a simultaneously conversion of hemicellulose of 84% (SCB_t #9, #10 to 88% (SCB_t #3, #4) was achieved, in contrast with the 21% and 12% attained with untreated SCB, respectively. In fact, using a pretreaded biomass from either one of these selected pretreatment conditions, an efficient hydrolysis up to 91% of the total polysaccharides was attained generating a sugar-rich hydrolysate with 44 - 46 g L^{-1} total sugars from 5% (w/v) of biomass loading (SCB_t #9, #10 and SCB_t #3, #4, respectively), in contrast with the ~18% of hydrolysis yield and an hydrolysate with only ~9 g L^{-1} total sugars achieved using directly untreated SCB. So, using either the KOH 3.25% - 13 min or the KOH 10% - 35 min as the pretreatment condition for subsequent SCB_t hydrolysis a yield of total fermentable sugars of about 866 to 880 mg sugar/g biomass was attained from pretreated SCB, which correspond to an increase of 6.71 to 6.82 times relatively to the yield of only 129 mg sugar/g raw biomass (Fig. 6), despite the great loss of solids inherent to the pretreatment process (41-54% loss, Table 1). The main difference in the sugar-rich hydrolysates obtained from these two SCB_t is the ratio of glucose/xylose derived from the conversion of cellulose/xylan (see Table 1). The pretreatment with KOH 10%, 35 min will be an advantage if the highest ratio glucose/xylose is intended in the subsequent biomass hydrolysis, since the corresponding SCB_t presented about 80% of cellulose within 97% of total polysaccharides content (cellulose + hemicellulose). Moreover, the autoclaving time of this pretreatment can still be reduced at least to 13 min as in the treatment with KOH 3.25%, given the limited influence of time as demonstrated in the experimental design response surface data (Figs 1-4).

The total fermentable sugars yield observed in this study (866-880 mg sugar/g SCB_t), achieved using KOH as the alkali agent for SCB pretreatment, was a little higher than that achieved by Rezende et al.[16] in their two-step pretreatment of SCB (H_2SO_4 1% + NaOH 1%). In their pretreatment, 814 mg/g SCB_t was attained, which corresponded to 99.8% of cellulose conversion and respectively to 96.1% of total polysaccharides conversion. In fact, comparing the total polysaccharide conversion yield with that obtained in this study (96.1% *vs* 91%), the difference observed is mainly related with the total polysaccharides content in pretreated biomass (847 mg/g *vs* 952-967 mg/g, Fig. 6). Although the sugars generation be lower (814 mg/g), the total polysaccharides content was also lower (847 mg/g) resulting in higher conversion yield (96.1%). This highlights the need of uniform results for feasible comparisons between pretreatment effectiveness towards sugars generation.

Since the goal of a pretreatment is to obtain the highest sugar generation from the lignocellulosic biomass, the most effective treatments should be those which permit to accomplish that goal, evidently accounting the overall costs associated to the process. The advantage of performing enzymatic hydrolysis at high solids loading in comparison to low or moderate loads is the resulting high sugar for further biotechnological processes. Therefore, combining pretreatment and subsequent hydrolysis at the highest loadings possible (>15%) is important to turn the bioprocess cost-effective. In this context, Martins et al.[45], after enzymatic hydrolysis of 10% up to 20% solids of a SCB biomass, prior pretreated at the same solids concentration with 7% H_2O_2 at pH 11.5 adjusted with NaOH (*i.e.* 7% AHP – Alcaline hydrogen peroxide, 90ºC, 1h), obtained a glucose concentration from 55.2 ± 0.50 g L^{-1} (10% solids) up to 82.25 ± 0.43 g L^{-1} (20% solids, fed-batch). Hence, the sugar concentration in the final hydrolysate was highly increased, which is crucial when the goal is an economical viable ethanol production, although the overall conversion yield decreased (76% to 57%), corresponding to a lower net sugar generation from the biomass (498 mg/g SCB_{AHP} to 373 mg/g SCB_{AHP}, considering the AHP treated SCB with 65.5% cellulose).[45] Similarly, Ramos et al.,[46] from an enzymatic hydrolysis, of 20% solids of a phosphoric acid-impregnated steam-treated SCB, attained a hydrolysate with 76.8 g L^{-1} of glucose equivalents, corresponding to a glucan conversion of only 69.2% (*i.e.* net sugar generation of ~381 mg/g SCB_{SE}, considering the ~55% of glucan content in SCB_{SE}).

Indeed, both pretreatments selected in this study, KOH 10% - 35 min and KOH 3.25% - 13 min (Fig. 6), permitted to obtain treated biomass with >95% total polysaccharides, without inhibitors (HMF, furfural), which enhanced the subsequent enzymatic attack (91% conversion yield). Therefore, these two pretreatment have high potential to be improved in further optimization procedure considering the increase of the solids loading both in pretreament itself and in following enzymatic hydrolysis. Moreover, sugar concentration within hydrolysis can be also significantly increased by changing the feeding strategy to fed-batch (fed-batch hydrolysis).[45] These further trials will contribute to turn the SCB delignification with KOH economical viable, since a net balance must be carry out between costs (e.g. KOH cost) and effectiveness. An effective pretreatment must preserve the maximal carbohydrates and avoid the formation of inhibitors towards further enhanced digestability and consequently higher bioconversion yield.

Moreover, Sharma et al.[30] also have demonstrated the potential of KOH pretreatment over NaOH pretreatment towards the subsequent fermentable sugar production from switchgrass, increasing the yield of total reducing sugars from 453 mg/g biomass[47] to 582 mg/g biomass, from a treated biomass with about 662 mg total polysaccharides/g (92 ± 9% of conversion yield).

In overall, a pretreated SCB containing >95% of total polysaccharides available for further sugar generation, is a promising biomass for the production of sugar-rich hydrolysates using adequate enzyme cocktails, either towards 2G bioethanol production by selecting an ethanologenic yeast able to ferment both xylose and glucose, such as *Pichia stipitis*[48,49] or other yeasts more recently designed or described as xylose fermenting strains,[50-53] or towards other added-value bioproducts production.[5,10]

D. Scanning electron microscopy

Images obtained by scanning electron microscopy on the surfaces of raw SCB (untreated sample) in comparison with SCB treated with KOH 10% for 35 minutes in autoclave revealed the morphological

changes induced by the pretreatment on bagasse (Fig. 7). In raw SCB, the fibres are intact, without any alteration or disruption of the cell wall (Figs. 7a and 7b). In SCB_t (Figs. 7c and 7d), it can be observed the change in fibre morphology with a significant unstructuring as a consequence of the delignification pretreatment with KOH 10% for 35 minutes applied to the SCB. A similar SEM surface image was also observed on SCB treated only with KOH 3.25%, but with an identical delignification (data not shown). The alkaline pretreatment increased the SCB reaction area and the fibre disruption contributed for the remarkable improvement observed in the subsequent enzyme saccharification of SCB_t (Fig. 5). The lignin removal from SCB favoured the enzyme access to the polysaccharides fraction through a less resistant cell wall.

IV. Conclusions

KOH was proven to be a suitable pretreatment for SCB in order to enhance its fibres deconstruction, namely through lignin removal, boosting the subsequent enzymatic hydrolysis. In fact, an effective pretreatment must minimize the polysaccharides loss, inhibit toxic byproduct formation (e.g. furfural, HMF) and enhance enzyme efficiency. The pretreatment of SCB with KOH, designed in this study, produced SCB_t samples with an average cellulose level up to 80%, within 97% of total polysaccharides, without inhibitors (furfural, HMF) and with ≤5% lignin in one-step treatment. Based on the greatest delignification (70-74%) and polysaccharides availability (95-97%) after pretreatment, SCB_t samples from two pretreatment conditions (KOH 3.25% - 13 min; KOH 10% - 35 min) were selected and further hydrolysed for fermentable sugar production. High sugar yields were obtained from both pretreated samples (866 to 880 mg sugar/g biomass, respectively) in contrast with the 129 mg sugar/g raw biomass obtained from untreated SCB. These results demonstrate the effectiveness of alkali pretreatment with KOH, which improved the overall digestibility of raw SCB polysaccharides from 18% up to 91%, generating a sugar-rich hydrolysate up to 46 g L^{-1} total fermentable sugars from a 5% of biomass loading. However, the harsh alkali treatment (KOH 10%) is preferred for the highest glucose/xylose ratio in the final sugar-rich hydrolysate. The use of hydrolysates obtained from SCB_t as carbon source for industrial purposes may provide a sustainable and economic solution for the production of bio-based added-value products, such as 2G bioethanol. However, further overall optimization, considering both pretreatment conditions (e.g. lesser autoclaving time; treatment temperature reduction; higher solids loading), and enzyme loading during the subsequent hydrolysis, using fed-batch feeding strategy for higher solids loading also, is still required towards a cost-effective biotechnological process that may be integrated within a sugar production factory or 1G bioethanol factory.

Acknowledgements

The present work, performed at LNEG, was partially funded by FEDER funds through POFC-COMPETE and by national funds through FCT (Fundação para a Ciência e a Tecnologia) in the scope of project Carbon4Desulf (FCOMP-01-0124-FEDER-013932) and also through FCT in the scope of the bilateral cooperation project FCT/CNR - Italia128585955528141. The authors also would like to acknowledge the technical support in biomass chemical characterization by M. Céu Penedo.

Notes and references

[1]LNEG – Laboratório Nacional de Energia e Geologia, IP, Unidade de Bioenergia, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal (*Corresponding authors: *susana.alves@lneg.pt*; *luis.alves@lneg.pt*)

[2]UENF - Universidade Estadual do Norte Fluminense Darcy Ribeiro, LTA-CCTA, Campos dos Goytacazes, RJ, Brazil

1. K. Hofsetz and M.A. Silva, *Biomass Bioenerg*., 2012, **46**, 564–576.
2. G.J.V. Betancur and N.J. Pereira, *Electron J. Biotechn..,* 2010, **13**, 1–9.
3. C.A. Cardona, J.A. Quintero and I.C. Paz, *Bioresource Technol*., 2010, **101**, 4754–4766.
4. F. Peng, J.L. Ren, F. Xu, J. Bian, P. Peng and R.C. Sun, *J. Agr. Food Chem.*, 2010, **58**, 1768–1776.
5. A. Pandey, C.R. Soccol, P. Nigam and V.T. Soccol, *Bioresource Technol.*, 2000, **74**, 69–80.
6. M. Balat and H. Balat, *Appl. Energ.*, 2009, **8**, 2273–2282.
7. C. Ververis, K. Georghiou, D. Danielidis, D.G. Hatzinikolaou, P. Santas, R. Santas and V. Corleti, *Bioresource Technol.*, 2007, **98**, 296–301.
8. M. Pauly and K. Keegstra, *Plant J.*, 2008, **54**, 559–568.
9. J.G. Linger, D.R. Vardona, M.T. Guarnieri, E.M. Karpa, G.B. Hunsinger, M.A. Franden, C.W. Johnson, G. Chupkad, T.J. Strathmannn, P.T. Pienkos and G.T. Beckham, *PNAS*, 2014, **111**, 12013–12018.
10. A.K. Chandel, S.S. da Silva, W. Carvalho and O.M. Singh, *J. Chem. Technol. Biotechnol.*, 2012, **87**, 11–20.
11. N. Jayapal, A.K. Samanta, P.K. Atul, S. Senani, K.P. Suresh and K.T. Sampath, *Ind. Crops Prod.*, 2013, **42**, 14–24.
12. R. Rodriguez-Vazguez, G. Villanuevaventura and E. Riosleal, *Bioresource Technol.*,1992, **39**, 17–22.
13. P. Kumar, D.M. Barrett, M.J. Delwiche and P. Stroeve, *Ind. Eng. Chem. Res.*, 2009, **48**, 3713–3729.
14. R.C.L.B. Rodrigues, G.J.M. Rocha, D. Jr Rodrigues, H.J.I. Filho, M.G.A. Felipe and A. Jr Pessoa, *Bioresource Technol.*, 2010, **101**, 1247–1253.
15. A. Goshadrou, K. Karimi and M.J. Taherzadeh, *Ind. Crops Prod.,* 2011, **34**, 1219–1225.
16. C.R. Rezende, M.A. Lima, P. Maziero, E.R. Azevedo, W. Garcia and I. Polikarpov, *Biotechnology for Biofuels,* 2011, **4**, 54.
17. C. Krishnan, L.C. Sousa, M. Jin, L. Chang, B.E. Dale and V. Balan, *Biotechnol. Bioeng.*, 2010, **107**, 441–450.
18. C. Carrasco, H.M. Baudel, J. Sendelius, T. Modig, C .Roslander, M. Galbe, B. Hahn-Hägerdal, G. Zacchi and G. Lidén, *Enzyme Microb. Technol.*, 2010, **46**, 64–73.
19. J.R. Monte, M. Brienzo and A.M.F. Milagres, *Appl. Energ*., 2011, **88**, 403–408.
20. E.P. Dagnino, E.R. Chamorro, S.D. Romano, F.E. Felissia and M.C Area, *Ind. Crops Prod.*, 2013, **42**, 363–368.
21. N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple and M. Ladisch, *Bioresource Technol.*, 2005, **96**, 673–686.
22. M.O.S. Dias, M.P. Cunha, R. Maciel Filho, A. Bonomi, C.D.F. Jesus and C.E.V. Rossell, *J. Ind. Microbiol. Biotechnol*., 2011, **38**, 955–966.
23. M.E. Himmel, S.Y. Ding, D.K. Johnson, W.S. Adney, M.R. Nimlos, J.W. Brady and T.D. Foust, *Science*, 2007, **315**, 804–807.
24. Y.P. Zhang and L.R. Lynd, *Biotechnol. Bioeng.*, 2004, **88**, 797-824.
25. P. Alvira, E. Tomás-Pejó, M. Ballesteros and M.J. Negro, *Bioresource Technol*., 2010, **101**, 4851–4861.

26. R.A. Silverstein, Y. Chen , R.R. Sharma-Shivappa, M.D. Boyette and J. Osborne, *Bioresource Technol*., 2007, **98**, 3000–3011.

27. N.I. Iberahim, J.M. Jahim, S. Harun, M.T.M. Nor and O. Hassan, *IJCEA*, 2013, **4**, 101–105.

28. R. Rodriguez-Vazguez and D. Diazcervantes, *Bioresource Technol.*, 1994, **47**, 159–164.

29. L.G.A. Ong, C. Chuah and A.L. Chew, *J. Applied Sci*., 2010, **10**, 2608–2612.

30. R. Sharma, V. Palled, R.R. Sharma-Shivappa and J. Osborne, *Appl. Biochem. Biotechnol*., 2013, **169**, 761–772.

31. E. Raymundo-Piñero, P. Azaïs, T. Cacciaguerra, D. Cazorla-Amorós, A. Linares-Solano and F. Béguin, *Carbon*, 2005, **43**, 786–795.

32. V.S. Chang and M.T. Holtzapple, *Appl. Biochem. Biotechnol*., 2000, **84–86**, 5–37.

33. R. Kumar and C.E. Wyman, *Biotechnol. Bioeng*., 2009, **103**, 252–267.

34. M. Asgher, Z. Ahmad and H.M.N. Iqbal, *Ind. Crops Prod.*, 2013, **44**, 488–495.

35. D.H. Doehlert, Uniform shell designs. *Appl. Stat.*, 1970, **19**, 231–239.

36. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker, Technical Report NREL/TP-510-42618, 2011, 18p.

37. L. Maurelli , E. Ionata, F. La Cara and A. Morana, *Appl. Biochem. Biotechnol*., 2013, **170**, 1104–1118.

38. W.E. Kaar, C.V. Gutierrez and C.M. Kinoshita, *Biomass and Bioenerg.*, 1998, 14, 277–287.

39. M. Camassola and A. J. P. Dillon, *Energ. Fuel.*, 2012, **26**, 5316–5320.

40. M. Tutt, T. Kikas, H. Kahr, M. Pointner, P. Kuttner and J. Olt, *Agron. Res.*, 2014, **12**, 417–424.

41. K.C. Nlewem and M.E. Thrash Jr, *Bioresource Technol.*, 2010, **101**, 5426–5430.

42. Z. Wang, D.R. Keshwani, A.P. Redding and J.J. Cheng, *Bioresource Technol.*, 2010, **101**, 3583–3585.

43. T.P. Silva, S.M. Paixão, A.V. Teixeira, J.C. Roseiro and L. Alves, *J. Chem. Technol. Biotechnol*., 2013, **88**, 919–923.

44. S.N. Deming and S.L. Morgan, 1st ed., Elsevier Science Publishers, 1987.

45. L.H.S. Martins, S.C. Rabelo and A.C. Costa, *Bioresource Technol*., 2015, **191**, 312–321.

46. L.P. Ramos, L. Silva, A.C. Ballem, A.P. Pitarelo, L.M. Chiarello and M.H.L. Silveira, *Bioresource Technol*., 2015, **175**, 195–202.

47. J. Xu, J. J. Cheng, R.R. Sharma-Shivappa and J.C. Burns, *Energ. Fuel.*, 2010, **24**, 2113–2119.

48. S. Marques, L. Alves, J.C. Roseiro and F.M. Gírio, *Biomass Bioenerg.*, 2008, **32**, 400–406.

49. C.F. Huang, T.H. Lin, G.L. Guo and W.S. Hwang, *Bioresource Technol.*, 2009, **100**, 3914–3920.

50. R.E. Hector, B.S. Dien, M.A. Cotta, N. Qureshi, *J. Ind. Microbiol. Biotechnol.*, 2011, **38**, 1193–1202.

51. L. Diao, Y. Liu, F. Qian, J. Yang, Y. Jiang and S. Yang, *BMC Biotechnology*, 2013, **13**, 110.

52. M.M. Demeke, H. Dietz, Y. Li, M.R. Foulquié-Moreno, S. Mutturi, S. Deprez, T.D. Abt, B.M. Bonini, G. Liden, F. Dumortier, A. Verplaetse, E. Boles and J.M. Thevelein, *Biotechnology for Biofuels*, 2013, **6**, 89.

53. C.G. Morais, R.M. Cadete, A.P. Uetanabaro, L.H. Rosa, M.A. Lachance and C.A. Rosa, *Fungal Genet. Biol.*, 2013, **60**, 19–28.

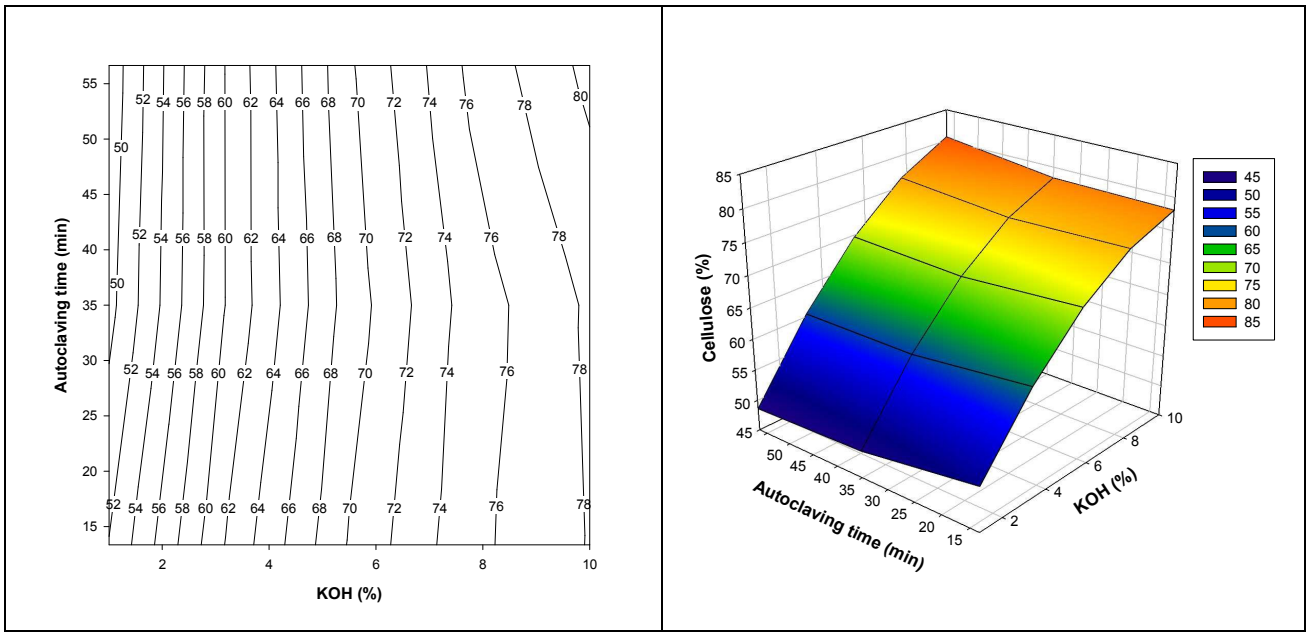


Fig. 1 Response surface (% cellulose) for the factors KOH concentration (1-10%) and autoclaving time (10-60 minutes), showing the cross-influence on cellulose level in SCB_t.

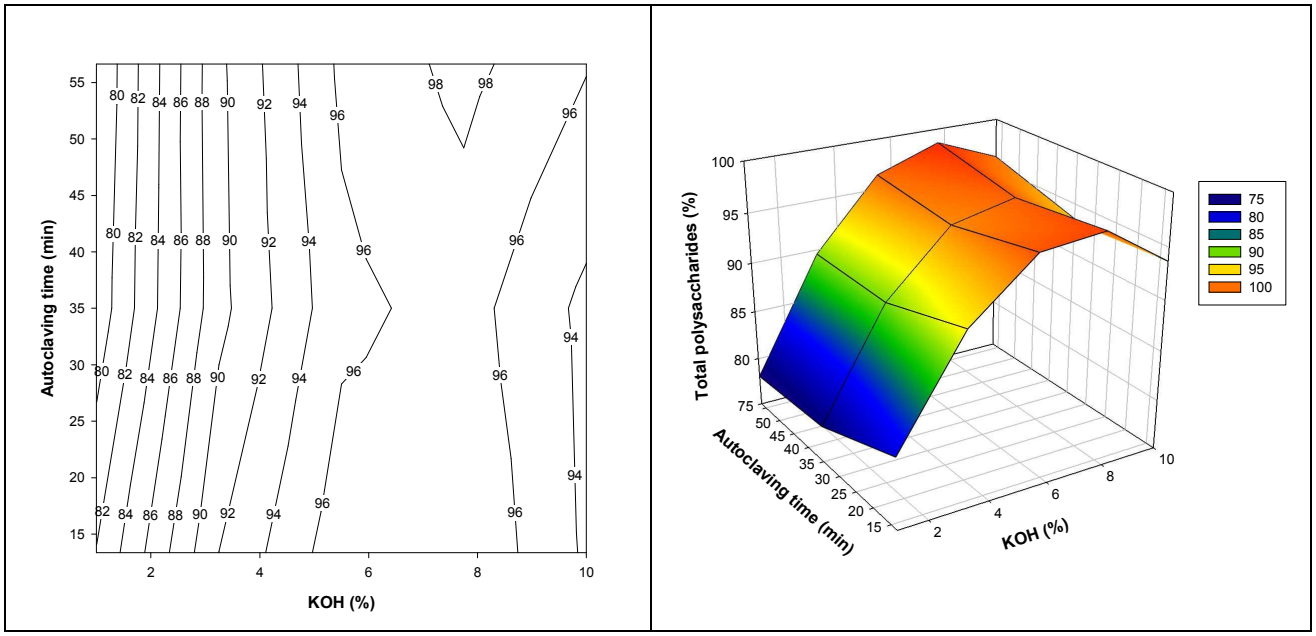


Fig. 2 Response surface (% total polysaccharides) for the factors KOH concentration (1-10%) and autoclaving time (10-60 minutes), showing the cross-influence on total polysaccharides level in SCB_t.

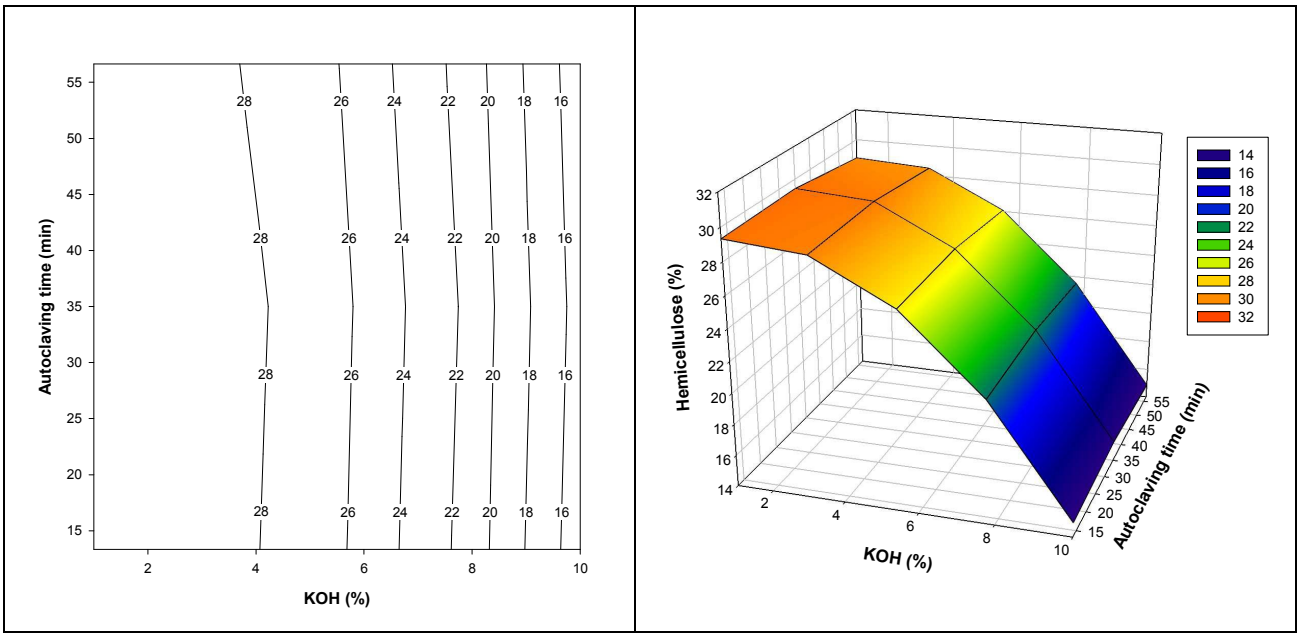


Fig. 3 Response surface (% hemicellulose) for the factors KOH concentration (1-10%) and autoclaving time (10-60 minutes), showing the cross-influence on hemicellulose level in SCB_t.

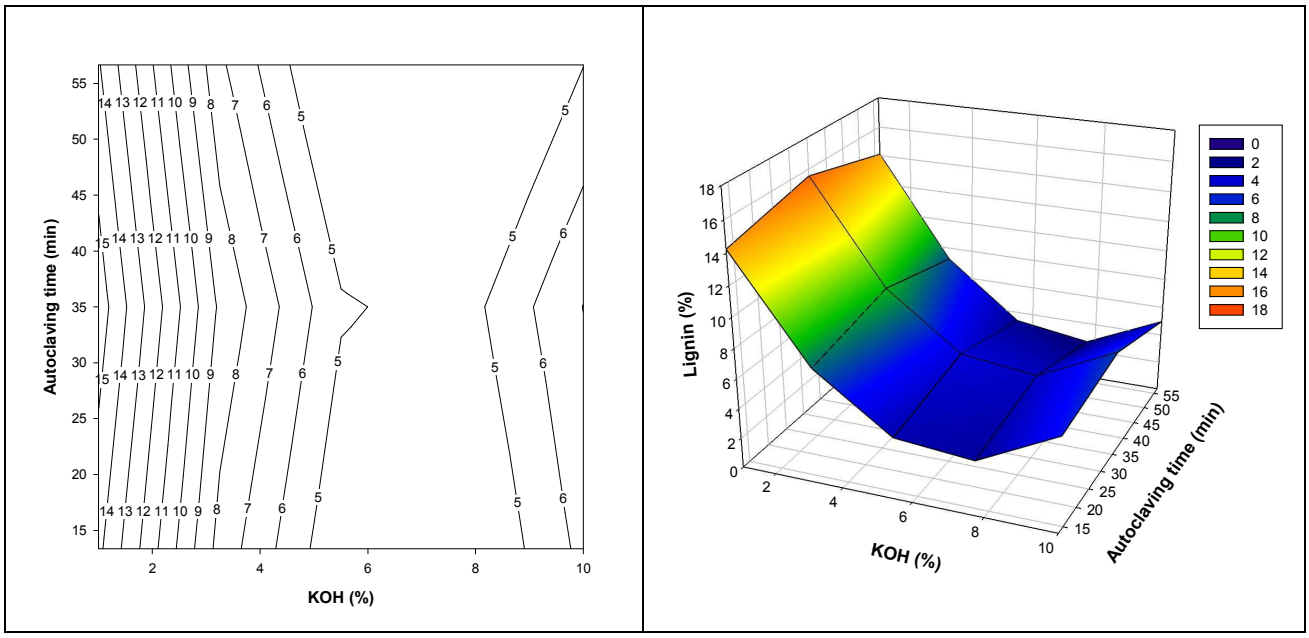


Fig. 4 Response surface (% lignin) for the factors KOH concentration (1-10%) and autoclaving time (10-60 minutes), showing the cross-influence on lignin content in SCB_t.

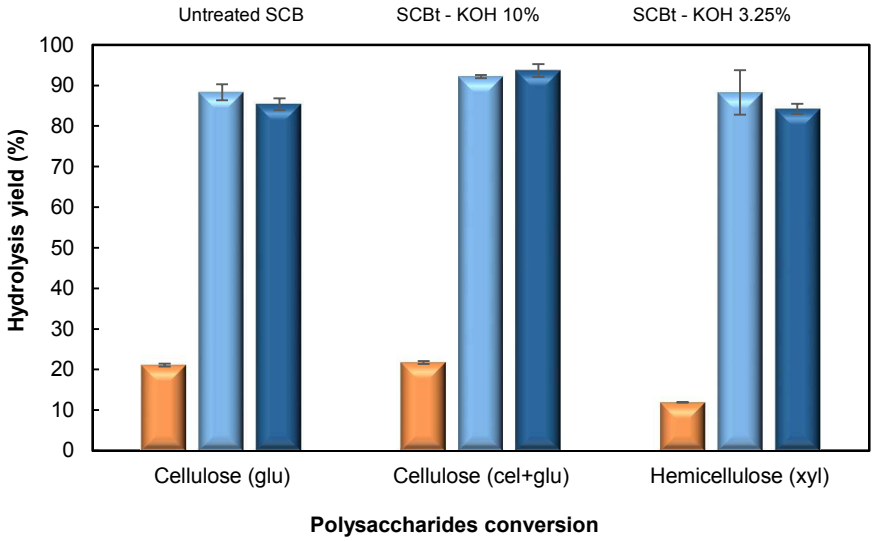


Fig. 5 Cellulose and hemicellulose conversion (%) for untreated SCB and SCB_t from two pretreatment conditions (KOH 3.25% - 13.35 min; KOH 10% - 35 min) after 72 hours of enzymatic hydrolysis. The error bars are the errors from the average values of duplicate saccharification assays (samples #3, #4 and samples #9, #10 were used as replicates of the two $SCB_{t,,}$ respectively from KOH 10% and KOH 3.25% pretreatments). Cel – cellobiose, glu – glucose and xyl – xylose, are the sugars released during hydrolysis of cellulose and hemicellulose. Cellulose (glu) – value for cellulose hydrolysed based in the amount of glucose released; Cellulose (cel+glu) – value for cellulose hydrolysed based in the amount of glucose and cellobiose released and Hemicellulose (xyl) – value for hemicellulose hydrolysed based in the amount of xylose released.

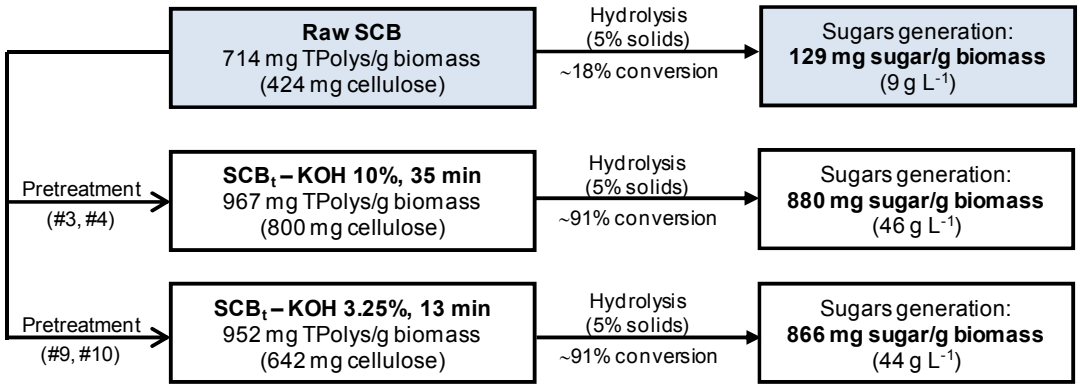


Fig. 6 Sugars generation from SCB (mg sugar/ biomass), raw material (untreated) *versus* pretreated biomass (SCB_t) from two pretreatment conditions (KOH 3.25% - 13.35 min; KOH 10% - 35 min), within 72 h of hydrolysis of 5% solids loading with the enzymatic cocktail at 50 °C, 150 rpm. Samples #3, #4 and samples #9, #10 were used as replicates of the two SCB_t respectively from KOH 10% and KOH 3.25% pretreatments. TPolys: total polysaccharides (cellulose + hemicellulose); Sugars = total reducing sugars (glucose + xylose + cellobiose).

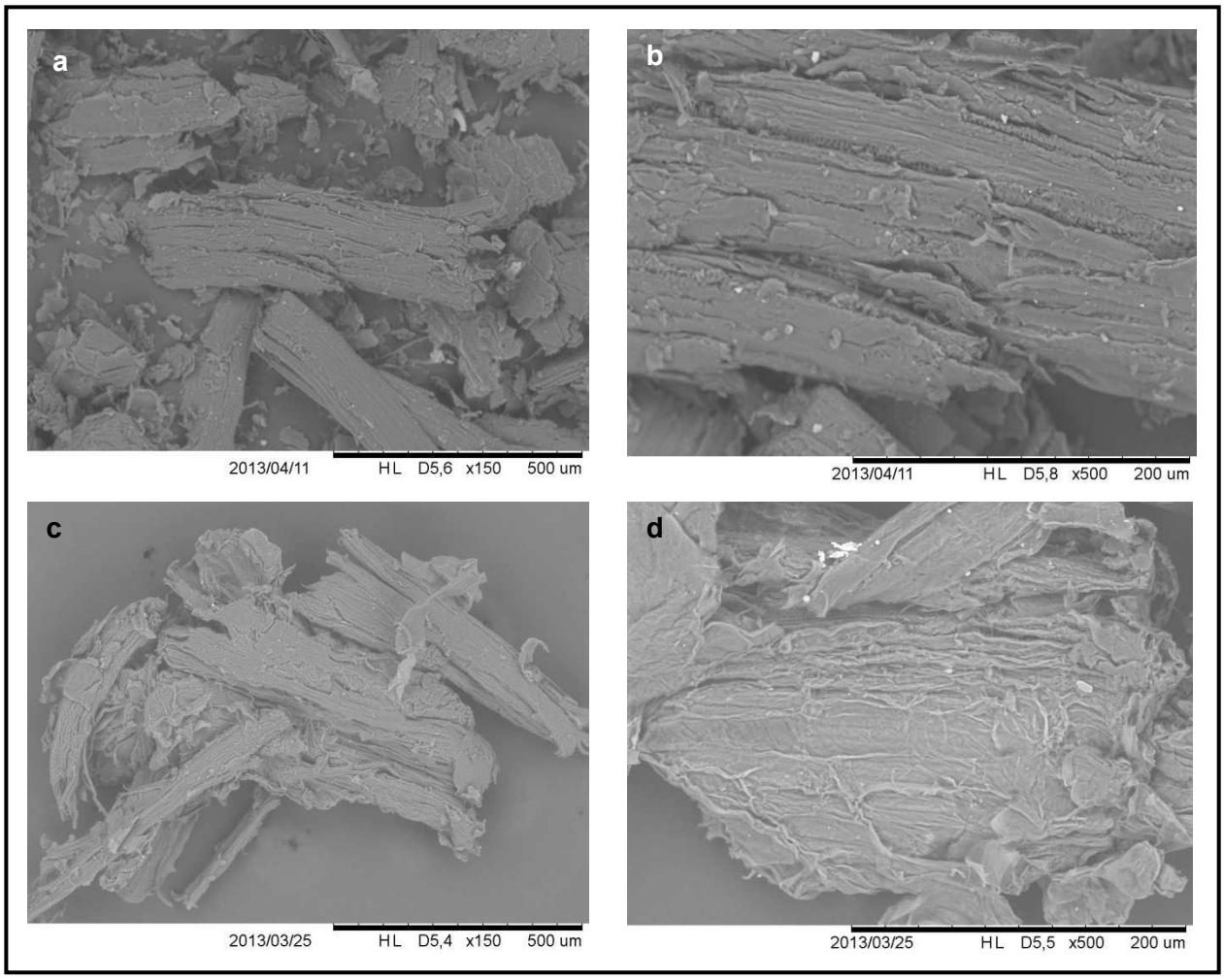


Fig. 7 Scanning electron microscopy surface images of raw SCB (a, b) *versus* SCB treated with KOH 10%, for 35 minutes at 121 ºC (c, d).

Table 1 Chemical characterization of SCB submitted to different delignification pretreatments according to the statistical design *versus* SCB

Test (#)	KOH (%)	Time (minutes)	Cellulose[a] (%)	Hemicellulose[a] (%)	T. Polysaccharides[b] (%)	Lignin[a] (%)	Ash (%)	Acetic Acid (%)	Pretreatment yield[c] (%)
Untreated	---	---	42.43	28.96	71.39	18.61	1.47	4.35	100.0
1	5.5	35	68.92	26.56	95.48	5.32	0.18	3.34	51.59
2	5.5	35	68.81	26.55	95.36	4.93	0.16	2.69	
3	10	35	79.86	19.34	99.19	4.53	0.31	5.03	45.55
4	10	35	80.42	13.81	94.24	5.24	0.34	0.15	
5	1	35	47.31	27.73	75.04	17.97	1.07	3.99	76.16
6	1	35	47.57	28.32	75.89	17.43	0.63	1.48	
7	7.75	56.65	73.59	20.43	94.02	4.97	0.12	0.23	46.24
8	7.75	56.65	75.48	21.32	96.80	4.62	0.10	4.25	
9	3.25	13.35	64.05	30.51	94.55	5.47	0.55	0	58.96
10	3.25	13.35	64.38	31.45	95.83	5.51	0.51	0	
11	7.75	13.35	72.85	20.61	93.46	6.04	0.53	3.05	48.68
12	7.75	13.35	74.26	21.49	95.75	5.54	0.54	0.22	
13	3.25	56.65	62.34	30.59	92.93	4.94	0.42	0	56.19
14	3.25	56.65	62.13	30.49	92.61	5.20	0.52	2.23	

[a]Responses of the experimental design (Yi)

[b]Total Polysaccharides = Cellulose + Hemicellulose

[c]Solid recovery

Note: None of the SCB or SCB_t (1-14) samples contained furfural or HMF. All the values are an average of at least two replicates (Coefficient of variation: 0.5-5.0%).

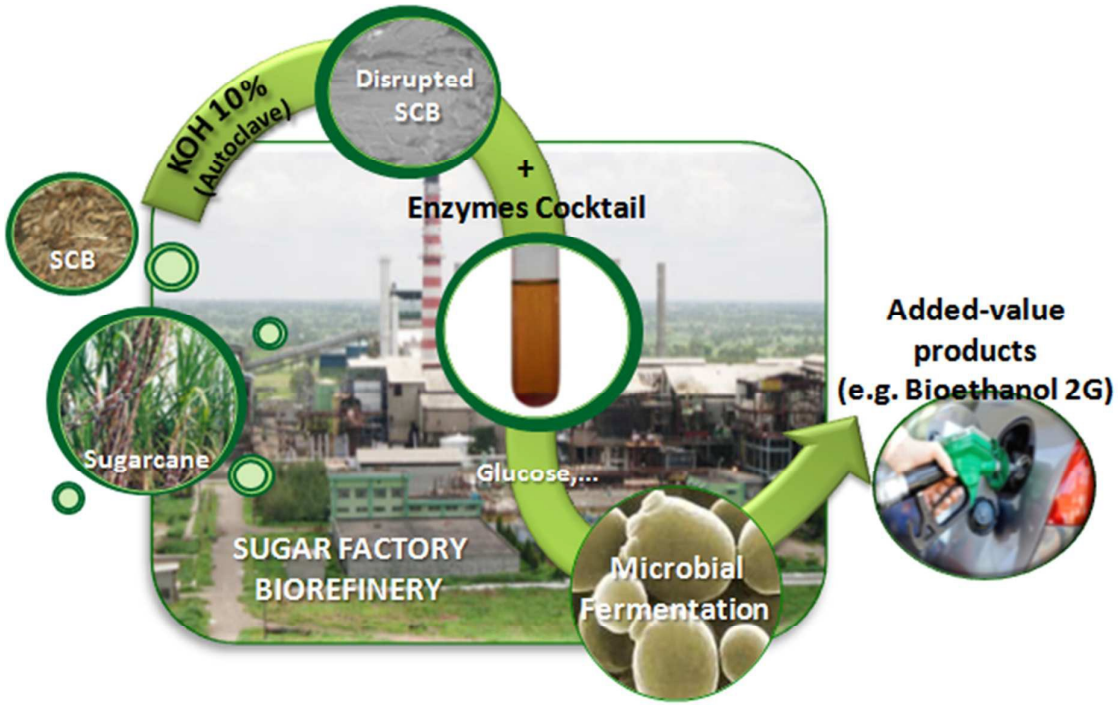
Table 2 Parameters of polynomial models representing the responses studied. (β0, response at the center of the experimental domain; β1 and β2, parameters of factors 1 and 2, respectively; β12, the parameter of interaction of the factors 1 and 2; β11 and β22, self-interaction parameters of the factors 1 and 2, respectively)

	Model	Cellulose	Hemicellulose	Total Polysacchar.	Lignin
Model Parameters	β0	68.88	26.56	95.44	5.13
	β1	14.51	-7.08	7.42	-4.27
	β2	-0.29	-0.18	-0.47	-0.41
	β12	1.71	0.15	1.86	-0.33
	β11	-5.09	-4.26	-9.34	6.16
	β22	1.37	0.49	1.85	-1.84
Model Validation (Fischer Test)	Effectiveness of the parameters	48.77	14.06	6.15	6.16
	Significance Level	F(5,8), α = <0.01	F(5,8), α = 0.01	F(5,8), α = 0.01	F(5,8), α = 0.01
	Lack of fit	93.48	9.27	43.12	5652.34
	Significance Level	F(1,7), α = <0.01	F(1,7), α = <0.1	F(1,7), α = <0.01	F(1,7), α = <0.01
R^2	Coefficient of multiple determination	0.97	0.90	0.79	0.79

Graphical Abstract

Sugarcane bagasse delignification with potassium hydroxide for enhanced enzymatic hydrolysis

S.M. Paixão, S.A. Ladeira, T.P. Silva, B.F. Arez, J.C. Roseiro, M.L.L. Martins, L. Alves



Treated SCB biomass was further hydrolysed using an enzymatic cocktail demonstrating the effectiveness of pretreatment towards sugar-rich hydrolysate production for biotechnological applications.