Influence of temperature and soda concentration in a thermo-mechano-chemical pretreatment for bioethanol production from sweet corn co-products

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A B S T R A C T

A continuous process combining an alkaline pretreatment, neutralization and injection of enzymes within a twin screw extruder was previously implemented and demonstrate industrial potential. The present work focuses on the investigation of the effects of alkali and temperature during the alkaline pretreatment of sweet corn co-products (SCC) for the production of fermentable sugars with a lower chemical input. Study of NaOH/SCC and internal temperature was performed in ranges of 4–8% (w/w) and 50–170 °C in a laboratory scale twin screw extruder. Analysis of carbohydrates and lignin of the pretreated biomass was performed and the filtration efficiency was also monitored through extrudate dry matter and filtrate mineral matter. The carbohydrate accessibility and process performances were studied by the enzymatic hydrolysis of the extrudate. Increasing temperature reinforces the effects of soda on solubilization of hemicelluloses, thus a hemicelluloses removal reach more than 50%. At optimal conditions, the cellulose-rich substrate after enzymatic hydrolysis achieve a glucose released of 70%, with glucose and xylose yields of 250 g per 1Kg of dry SCC.

1. Introduction

The rise in global population necessities and upsurge in food and energy demands lead to the current environmental crisis. For instance, an increment in Green House Gas emissions forecasts temperature rises and climate perturbations (Schleussner et al., 2016). Therefore, to ensure sustainable food security, preservation of soil quality, the biodiversity and water reserves need to be addressed (Godfray et al., 2010). Additionally, with the increasing scarcity of fossil fuel, development of sustainable alternatives for transport energy are required (Leggett and Ball, 2012; Solomon, 2010). Transport energy demand solutions comprise bioethanol from renewable resources (Zabed et al., 2017). Bioethanol from lignocellulosic feedstocks (non-food raw material), such as agricultural residues, provide lower GHG emissions and fossil energy demand. However, its environmental impact significantly depends on land use conditions (Cherubini and Ulgiati, 2010).

Lignocellulosic bioethanol is obtained by a sequential unit operations i.e.: pretreatment, saccharification, fermentation, and distillation (Zabed et al., 2016). At present, overcoming the technical barriers at each unit operation dictates the economic feasibility of the production process. Concerning the pretreatment phase, optimization to increase enzymatic hydrolysis and fermentation efficiency while maintaining low operational costs are the major challenges (Chiaramonti et al., 2012). The biomass complexity in its physiochemical structure limits the enzymatic performances to deconstruct the carbohydrate matrix (Himmel et al., 2007). Thus, various physical, chemical, physico-chemical and biological technologies have been applied to lignocellulose to enhance the enzymatic accessibility; (Alvira et al., 2010; Chiaramonti et al., 2012; Putro et al., 2016; Sun and Cheng, 2002). Currently, few technologies such as steam explosion, dilute acid, and alkali pretreatment have reached demonstration and commercialization phases, and their economic competitiveness are under study (IRENA, 2016).

Alkaline pretreatment technology has been extensively studied using different reagents, such as sodium hydroxide (Chen et al., 2013), ammonia (Prior and Day, 2008), potassium hydroxide ((Sharma et al., 2013)) and lime (Kaar and Holtzapple, 2000). These alkali catalysts are known to disrupt the ester bonds between lignin and hemicelluloses, thereby providing partial or total lignin solubilization, resulting in the production of sugar oligomers and polymers. Furthermore, alkaline addition diminishes cellulose crystallinity by swelling which increases the internal surface area. In terms of process, temperature pretreatment

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presents advantages such as an affordable cost, high conservation of cellulosic material, low enzymatic production and fermentation inhibitors such as HMF and furfural (Kim et al., 2016; Modenbach, 2013; Rodrigues et al., 2016).

Therefore, by coalescing alkaline pretreatment with twin screw extrusion, a continuous, adaptable and a high solid efficient process can be achieved (Vandenbossche et al., 2014). As a pretreatment, extrusion is characterized by shearing and pressure forces applied by the screw rotation, thus, leading to particle size reduction and fibrillation of the biomass (Zhang et al., 2011). The combined effects of extrusion with chemical pretreatment have been previously underlined: higher particle size reduction, solubilization of biomass components and sugar yields by enzymatic hydrolysis (Duque et al., 2017). In a recent study, Liu et al., 2018 compared alkaline pretreatment (AP) and alkaline twin-screw extrusion (ATSE) pretreatment on corn stover. According to the authors, mixing and chemical penetration in the extruder combined with the fibrillation enhance the lignin solubilization and therefore, the accessibility to cellulose and hemicelluloses of enzymes (Liu et al., 2018). Comparison of alkali and alkali extrusion on corn residues was also analyzed by Khatri et al. (2018). Results indicate a loss of hemicelluloses at the biomass surface with both alkaline pretreatments, but lower crystalline cellulose was detected in alkali-extruded biomass (Khatri et al., 2018)

Earlier studies have executed a continuous thermo-mechano-chemical-technology using twin screw extrusion (Vandenbossche et al., 2015) with successful pilot scale tests using six different biomass sources (Vandenbossche et al., 2016). This novel process includes different steps within the extruder, namely; alkaline pretreatment, neutralization, filtration, and enzymatic impregnation. Alkali pretreatment in the extruder prepares the biomass to have greater accessibility to the enzymes; whereas a neutralization phase acidifies the biomass and filtration step eliminates the yeast and enzymatic inhibitory compounds (salts and soluble molecules)(Deparis et al., 2017). The results from the previous studies demonstrate the efficacy of alkaline pretreatment and the potential of bioextrusion to reach a viable techno-economic process to produce bioethanol. Thereby, in the present work, the influence of temperature and alkaline concentration in reactive extrusion is analyzed using the design of experiments (DOE) with an objective to produce an accessible and cellulose-rich substrate with a lower chemical load. For this matter, the parameters determined by DOE were applied to extrusion using sweet-corn co-product.

2. Materials and methods

2.1. Materials

2.1.1. Feedstock

Dehydrated sweet corn (*Zea mays L. saccharata*) co-products (SCC) were obtained from industrial corn canneries and were provided by SARL Soupro+ (Castelmoron sur Lot, France). It was milled using a hammer mill fitted with a 6 mm screen.

2.1.2. Twin-screw extrusion

The extrusion process was performed using a twin-screw extruder (Evolum 25, Clextral, France), composed of 10 modules with a length of 100 mm each. The extruder is configured to combine three different processing steps: alkaline pretreatment, neutralization, and filtration of the biomass (Fig. 1). The screw diameter is 25 mm. Four types of screws were used: a trapezoidal double-thread screw used for the feeding zone, conveying double-thread, screws bilobe paddle and reversed pitch double-thread screws used to produce transport, mixing, and shearing effects, respectively, along with the different zones of the process. A heating band thermoregulated modules and cooled by water circulation. Two internal temperature probes were introduced, in module 3 to monitor the temperature of alkali impregnation and module 8 to ensure matter temperature below 50 °C A filter section consisting of six hemispherical dishes with conical holes was used on module 6 to enable the filtrate to be collected. Feedstocks were fed into the extruder's first module using a feeder model number KCL-KT20 (K-tron, Clextral, France). Two piston pumps (DKM SUPER J PP 6.35, DKM SUPER K PP 16, Clextral, France) were used to inject, respectively, alkaline solutions of sodium hydroxide (NaOH) and acid solutions of sulfuric acid (H2SO4).

The conditions of extrusion were based on Vandenbossche et al. (2016) study at pilot scale. In this previous study, the pretreatment was conducted using sodium hydroxide at 10% (w/w) and barrel temperature of 100 °C, the neutralization was carried out with phosphoric acid at 3.5% (w/w)((Vandenbossche et al., 2016)). In this case, the screw rotation of the laboratory scale extruder was set at 300 rpm. The feeder screw discharged continuously the sweet corn coproduct at 1.8 Kg/h to obtain 1.6 Kg of dry sweet corn co-product per hour. Both pumps of reagents were fixed to maintain liquid solid ratios of 1.0 (Kg/Kg) in alkaline site and 4.2 (Kg/Kg) in acid introduction which correspond to a flow rate of 1.65 kg/h for alkali solution and 5.80 Kg/h for sulfuric acid solution. The pH of pretreated biomass was controlled to ensure that biomass was neutralized. The pH of extrudate and filtrate aimed were 5 and 4 respectively. Both extrudate and filtrate samples were stored in plastic containers at −20 °C.

2.2. Pretreatment and experimental design

To study the influence of NaOH and temperature in the pretreatment, the NaOH loading per g of dry matter of sweet corn co-product and the internal temperature monitored by the probe 1 (TP1) were evaluated. Pretreatment conditions were inspired by a 2 factors Dohelert experimental design. The experimental design consisted of a center at 120 °C and 6% variation steps of 60 °C for temperature and 2% for NaOH/SCC. The responses analyzed were the composition of extrudate (insoluble fiber, celluloses, hemicelluloses and lignin), hemicelluloses removal, the filtration efficiency parameters (extrudate dry matter and filtrate dry matter) and the enzymatic hydrolysis (enzymatic conversion and sugar yield).

The statistical analyses were performed with NemrodW®. Once the data was collected, the responses were normalized, then fitted to the empirical equations (second order polynomial regression equations) for identification of main variables:

$$Y = a_O + a_1X_1 + a_2X_2 + a_{12}X_1X_2 + a_{11}X_1^2 + a_{22}X_2^2 \quad (3.1)$$

Where the response is Y, the variables X_1 and X_2 represent the normalized parameters, alkaline loading and the internal temperature measured, respectively. The responses are correlated to the center of the domain a_0, linear (a_1 and a_2), interactions (a_{12}) and quadratic (a_{11} and a_{22}) coefficients. The coefficients a_1 and a_2 corresponding to the main effects of NaOH/SCC and internal temperature respectively.

2.3. Analytical methods

2.3.1. Extrudate analysis

$$HR = ((H\ in\ initial\ SCC\ -\ H\ in\ extrudate))/(H\ in\ initial\ SCC) \times 100 \quad (3.2)$$

$$CY = ((C\ inextrudate)/(C\ initial\ SCC)) \times 100 \quad (3.3)$$

The French standard NF V 03–322 was used to determine moisture content and mineral content. An estimation of the three parietal constituents contained in the extrudates was performed using the ADF–NDF method from Van Soest and Vine (Soest et al., 1968). This method allows the quantification of insoluble fraction (IF), cellulose, hemicelluloses and lignin contained in the biomass. Insoluble fraction is obtained after neutral detergent wash and corresponds to the structural components in the fiber. All determinations were carried out in triplicate with a standard deviation < 1.5% for all measurements. Using

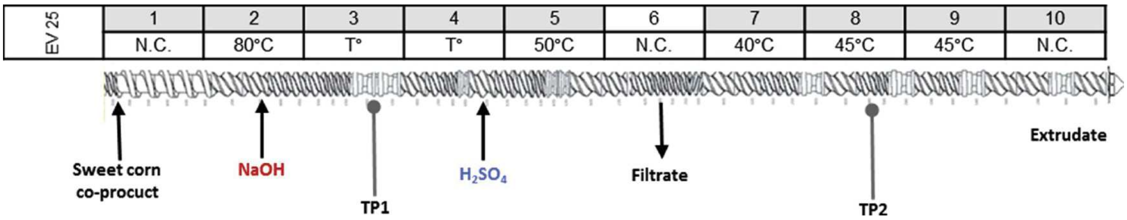

Fig. 1. Schematic diagram of the modular barrel of EV25 twin-screw extruders; N.C.: Non-controlled; TP: Temperature probe (to be viewed in color).

Table 1
Raw sweet corn characteristics: solid content, composition and enzymatic hydrolysis results.

	Units	Values		S.D.
Composition				
Dry matter	%	89.5	±	0.1
Minerals	% DM	5.9	±	0.3
Protein	% DM	8.5	±	0.22
Insoluble fiber	%DM	75.2	±	0.5
Cellulose	%IF (%DM)	40.8 (31.0)	±	0.8
Hemicelluloses	%IF (%DM)	53.1 (39.0)	±	
Lignin	%IF (%DM)	6.1 (5.0)	±	
Enzymatic hydrolysis at 48 h				
GR	% (g/g cellulose in SCC)	28.7	±	1.7
XR	% (g/g of hemicelluloses in SCC)	25.2	±	1.6
GY	% (g/g of dry SCC)	9.8	±	0.6
XY	% (g/g of dry SCC)	11.6	±	0.7

Note: DM: Dry Matter IF: Insoluble Fiber EH: Enzymatic Hydrolysis; GR: Glucose Released; XR: Xylose released; GY: Glucose Yield; XY: Xylose Yield.

these data and the extrudates flow rates, the mass balance of insoluble fiber, cellulose, hemicelluloses, and lignin were determined. The hemicellulose removal and the cellulose conservation rates were calculated by the following two equations (Eqs. (3.2) and (3.3)):

Where H stands for hemicelluloses and C to cellulose. The hemicelluloses and cellulose are expressed in mass.

2.3.2. Enzymatic hydrolysis

Enzymatic hydrolysis of extrudates were conducted at a consistency of 5% (W/W) on 0.5 *g* (extrudate dry matter) at 50 °C using a shaken hot-water bath and 25 mL glass Pyrex tubes for 48 h. Citrate-phosphate buffer (pH = 5) was added to maintain the pH at 5, and 0.02% sodium azide was used in the mixture to avoid microbial contamination. The commercial enzymatic cocktails used for the study were provided by Novozyme (Franklintong, USA), Cellic Ctec 2 and hemicellulases NS22002. The protein content of each cocktail was determined by the Lowry method (Biorad Protein assay kit), and cellulase activity was determined using the method reported by Ghose (Ghose, 2009). The protein content of Cellic Ctec 2 was 254.9 *g*/L and cellulase activity of 123.3 FPU/mL, and NS22002 protein content was 36.9 *g*/L. The Cellic Ctec 2 loading for saccharification assays was 15 FPU/g of the dry extrudate and was complemented NS22002 at 10% (protein basis).After 48 h of hydrolysis, the samples were diluted in 50 mL total volume. Afterwards, they were centrifuged and the supernatant was filtered through a 0.22 μm membrane. The hydrolysate was analyzed with the HPIC (High-performance ionic chromatography, Dionex, France) system using a Carbopac PA1 column. The efficiency of enzymes to release glucose and xylose was studied by glucose and xylose conversion rates, whereas the sugar yields evaluated the performance of the process to produce both monosaccharides using Eq. 3.4 & Eq. (3.5):

$$GR = m\ glucose\ released\ (corrected\ with\ blanks)\ /\ m\ C\ in\ extrudate * 1.1 * 100 \tag{3.4}$$

$$XR = m\ xylose\ released\ (corrected\ with\ blanks) / m\ H\ in\ extrudate * 1.1 * 100 \tag{3.5}$$

Where m is the mass of carbohydrate in grams, the cellulose (C) and hemicelluloses (H) in extrudate correspond to each carbohydrate contained in 0.5 g of dry extrudate. The 1.1 value corresponds to the conversion factor of glucan into glucose (180/162) and hemicelluloses into xylose and arabinose (150/132). SCC hemicelluloses are composed of 85% of xylan and 15% arabinose (data not shown). Therefore, in this study, XR only accounts as an indicator of the xylose produced.

$$Glucose\ Yield\ =\ m\ glucose\ released\ (corrected\ with\ blancks)\ /m\ dry\ raw\ SCC\ *\ 100 \tag{3.6}$$

$$Xylose\ Yield\ =\ m\ xylose\ released\ (corrected\ with\ blancks)\ /m\ dry\ raw\ SCC * 100 \tag{3.7}$$

Where m is the mass in grams. The mass of dry SCC was calculated as the ratio (Q_{ext}/Q_{SCC})*0.5, where Q_{ext} is the flow rate of oven-dry extrudate and Q_{SCC} is the flow rate of dry matter of sweet corn coproduct. Data presented in this paper are the averages of the results from triplicate experiments with standard deviation lower than 5%.

3. Results and discussion

3.1. Raw biomass analysis

The corn husk and cob form the sweet corn co-product. After harvesting, it was dried by the supplier to a final dry matter of 89.9%. Characteristics in terms of composition and enzymatic hydrolysis are presented in Table 1. SCC has a large lignocellulosic fraction, 75.2% formed by a large portion of carbohydrates, hemicelluloses (39%) and cellulose (31%) and a low lignin amount (5%). Soluble sugars content was analyzed. Glucose concentration was 0.013 g/g of biomass and traces of xylose, arabinose and galactose were detected at minor concentrations. Due to its high carbohydrate content and low lignin content, SCC is a promising substrate to produce bioethanol by enzymatic hydrolysis and co-fermentation. In terms of availability, canned industry in south-west of France reported production capacity between 280,000–390,000 t/year of SCC. As it is currently used for animal feed, it is considered as a waste, hence, a high potential feedstock for upcycling with economic viability.

3.2. Effect of soda loading and temperature

3.2.1. Extrudate and filtrate composition

According to Alvira et al., 2010 (Alvira et al., 2010), as bioethanol is potentially produced by co-fermentation of C5 and C6 sugars, an economically viable pretreatment is based on the cellulose and hemicelluloses recovery. Simultaneously, minimizing the effect of hemicelluloses and lignin as a barrier for saccharification. Alkaline pretreatments are generally known for the disruption of lignin structure and solvation of acetic groups in hemicelluloses, thus, producing solubilization of part of the lignocellulose (Kim et al., 2016). The analysis of extrudate composition at different conditions was conducted to monitor the effect of NaOH/SCC and temperature on the biomass carbohydrate content and lignin removal. The results are summarized in Table 2 and the statistical analysis in Table 3 and Fig. 2. Additionally, filtration efficiency is ensured by the mechanical force applied by the reverse

Table 2
Extrudate composition, mass balance indicators and filtrate mineral matter for all samples analyzed.

Sample	NaOH/SCC	Temperature	Extrudate composition					Mass balance indicators			Filtrate
			Solid content	IF	C	H	L	Q ext	CY	HR	MM
Units	% (w/w)	°C	%DM	%DM	%IF (%DM)	%IF (%DM)	%IF (%DM)	Kg/h	%	%	%DM
1	5.9	52	45.0	79.8	43.1 (34.4)	54.3 (40.5)	2.6 (4.9)	1.33	83.9	16.8	32.8
2	5.9	92	40.0	79.0	43.4 (34.2)	53.9 (40.3)	2.8 (4.4)	1.34	83.3	16.8	33.0
3	5.9	108	39.0	78.0	45.2 (35.3)	51.2 (37.9)	3.5 (4.8)	1.31	87.3	10.2	32.7
4	5.9	124	38.0	76.0	46.4 (35.5)	50.0 (36.7)	3.5 (4.3)	1.27	87.4	11.2	33.0
5	6.1	118	38.2	78.5	48.1 (37.7)	47.7 (37.0)	4.2 (3.7)	1.27	89.4	17.3	31.1
6	6.0	129	38.0	76.5	49.7 (38.0)	45.3 (34.7)	4.9 (3.2)	1.27	87.5	22.8	31.2
7	6.1	151	36.5	75.7	50.7 (35.3)	45.0 (34.3)	4.3 (3.6)	1.19	94.8	23.2	33.6
8	6.1	168	36.3	73.3	48.2 (38.4)	47.3 (34.1)	4.4 (3.2)	1.22	95.4	28.9	33.0
9	7.1	130	36.4	74.0	50.5 (37.4)	46.3 (34.3)	3.2 (2.4)	1.20	90.0	34.7	36.4
10	3.9	95	45.1	81.6	43.1 (35.2)	52.2 (42.6)	4.7 (3.8)	1.21	85.2	31.6	31.5
11	4.2	146	42.4	77.7	43.0 (33.5)	52.6 (40.9)	4.3 (3.4)	1.27	88.6	33.6	31.9
12	8.0	92	38.3	72.8	49.4 (36.0)	45.7 (33.3)	4.9 (3.5)	1.22	86.4	34.7	39.2
13	8.0	121	33.8	68.1	51.8 (35.2)	44.2 (30.1)	4.0 (2.7)	1.18	81.9	42.8	37.8
14	8.0	139	32.9	67.8	50.7 (34.4)	44.9 (33.6)	4.3 (2.9)	1.14	77.0	44.2	37.0

Note: SCC: Sweet Corn Co-product; IF: Insoluble Fiber; DM: Dry Matter; Qext: Extrutate **flow**; CY: Cellulose Yield; HR: Hemicelluloses Removal; MM: Mineral Matter.

screw in module 7, forming a dynamic plug that pressures the biomass. As the pretreatment deconstructs the biomass, the viscosity of the matter rises, affecting the dynamic plug stability and efficiency filtration. As filtration is less effective, moisture content in extrudate is higher and the mineral matter of filtrate is lower. Consequently, dry matter of both outputs were monitored, and results are shown in Table 4 and iso-response curves (Fig. 3).

The R^2 of the equation expresses the statistical accuracy of the fit. A significant R^2 means that the predicted values are close to the measured values. Statistical analysis performed on the extrudate composition demonstrates that the fit was accurate for IF, whereas for cellulose and hemicelluloses, R^2 of the models implies that only tendencies can be deducted.

Concerning lignin content in the extrudate, R^2 was not significant to be analyzed by the equation. For the IF, cellulose, and hemicelluloses, NaOH had a more significant impact than temperature as reflected by the p-values of their coefficients. Iso response curves of IF (Fig. 2**(a)**) display a strong influence of both parameters to diminish its value. When low NaOH and temperature are applied, IF fraction is essential. To disrupt the IF at 4% soda loading, a temperature higher than 150 °C is necessary. Nevertheless, the increasing soda loading disrupts IF, and its effect is reinforced by temperature.

Iso-response curves of H content (Fig. 2**(b)**) reveals a similar trend than IF, high H fraction is obtained with mild conditions and an increase in temperature and NaOH/SCC triggered its reduction. With temperatures below 90 °C, soda effect is only observed from 6%. As the temperature reaches 90 °C, NaOH solubilization of hemicelluloses is visible. Variations of H content due to NaOH are less noticeable at temperatures of 100–125 °C, with values between 45–52%. Values below 45% are attained with a temperature exceeding 125 °C and NaOH/SCC beyond 6%. To conclude, the temperature must surpass 50 °C to attain hemicelluloses solubilization. In the same way as IF, effect by soda is enhanced by an increase in temperature. Hence, reduction of IF is attributed to the partial solubilization of the hemicelluloses.

Considering the mass balance of the process, hemicelluloses removal (HR) rate from initial SCC was also analyzed (Fig. 2**(d)**). The HR is linear to the soda loading and the temperature attained. In the studied conditions, to reach more than 20% HR, both effects must be combined. To cite an instance, solubilization of hemicelluloses at 8% needs 50 °C to reach 20% removal, however, at 4% it needs more than 150 °C. Temperature needs to be attained to observe the NaOH effect on hemicelluloses. Although solubilization has occurred, a significant part of this fraction remained at the end of the process, considering that a minimum of 60% of the initial H is conserved, offering the possibility of improving ethanol yield by saccharification and co-fermentation of xylan.

The heterogeneous and amorphous structure of hemicelluloses makes it more susceptible to degradation by pretreatments than cellulose (Saha, 2003). By way of illustration, xylan losses were observed with Alkaline Twin Screw Extrusion pretreatment (ATSE process) by Liu et al. (2013) (Liu et al., 2013). During this process, biomass goes through a twin-screw extruder with 4 zones of compression as the alkaline solution is added. As soda loading was increased from 0.06 to 0.1 g/g of oven dry corn stover, the xylan loss raised from 21.7–39.3 %. Moreover, the study of soda concentration and barrel temperature using the present alkali-extrusion process by Duque et al. (2013) on barley straw, pinpointed that at the highest NaOH loading (i.e. 7.5% NaOH/g

Table 3
Statistical analysis of the effects of studied parameters on extrudate composition and mass balance indicators.

	Insoluble Fiber		Hemicelluloses		Cellulose		HR	
R^2	0.952		0.786		0.84		0.920	
Terms	Estimated	*p* value	Estimated	*p* value	Estimated	*p* value	Estimated	*p* value
a_0	78.04	< 0.01 ***	49.50	< 0.01 ***	46.81	< 0.01 ***	21.175	< 0.01***
a_1	−4.96	< 0.01 ***	−3.65	0.740 **	3.75	0.157 **	11.193	0.00477**
a_{11}	−2.63	0.514 **	−0.51	70.7	−0.22	83.1	6.987	2.16*
a_2	−3.29	0.0709 ***	−3.75	1.23 *	2.99	1.10 *	12.517	0.00541***
a_{22}	−1.31	21.2	1.21	52.6	−1.02	49.2	−0.220	2.40*
a_{12}	−0.89	52.8	−1.07	68.6	1.50	47.0	6.170	24.2

*indicate that term has a significant effect at 95% confidence interval.

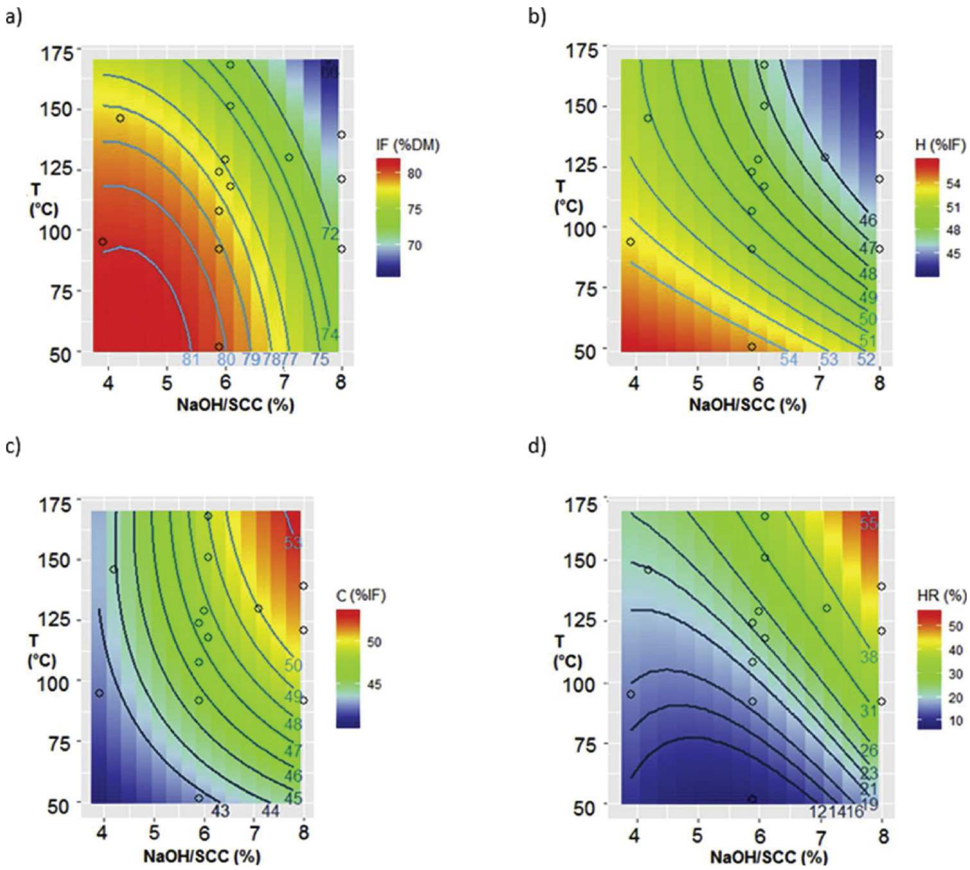

Fig. 2. Iso response curves indicating Insoluble Fiber (%DM) (a), hemicelluloses fraction (%IF) (b), cellulose fraction (%) (c) and hemicelluloses removal rate (%) (d) as function of the temperature (T) and NaOH/SCC ratio. The circles represent the experimental results. (To be viewed in color).

Table 4
Statistical analysis of the effect of the normalized parameters (temperature and NaOH/SCC) on extrudate (a) and filtrate mineral matter (b).

	Extrudate Dry matter		Filtrate mineral matter	
R^2	0.981		0.900	
Terms	Estimated	*p* value	Estimated	*p* value
a_0	38.76	< 0.01 ***	32.55	< 0.01***
a_1	−4.06	< 0.01 ***	3.46	0.0128***
a_{11}	1.01	2.83 *	2.31	0.686**
a_2	−4.11	< 0.01 ***	−0.33	70.5
a_{22}	1.94	0.646 **	0.55	56.0
a_{12}	−2.07	2.36 *	−1.32	32

*indicates that the term has a significant effect at 95% confidence interval.

dry barley straw), hemicellulosic fraction breaks down. Nevertheless, the impact of temperature in the conditions tested was not significant (Duque et al., 2013). Effect of the temperature on the hemicelluloses recovery after alkaline pretreatment of corn stover was reported by (Chen et al., 2013). The statistical analysis, presented for the recovery of hemicelluloses, indicated that temperature plays a key role in the solubilization of hemicelluloses. Pretreatments in literature are difficult to compare due to the variations on the substrate, extrusion profiles and process conditions. However, it is generally agreed that the hemicellulosic structure is disrupted by soda attack, while the temperature effect depends on the conditions applied.

Variation of the studied parameters demonstrates that hemicellulosic disruption by NaOH needs temperature, whereas lignin removal was detected at all conditions. At the mildest conditions (Sample 10), lignin concentration was already diminished to 4.7% instead of 6.1% in raw SCC. Nevertheless, delignification behavior is not regulated by the studied parameters, as the fitting had a low R^2, implying more parameters could be involved in lignin breakdown. Lignin restructuration after hydrothermal pretreatment on corn stem (Donohoe et al., 2008) and steam explosion on poplar holocellulose (Sannigrahi et al., 2011) were studied. The results exhibit that lignin fraction can undergo physico-chemical modifications such as re-condensation and formation of pseudo-lignin with carbohydrates occasioned by the temperature and pressure employed. In extrusion pretreatment, the effect of reverse screw elements on corncobs produced lignin re-distribution at the fiber surface, thus, blocking pores (Zheng et al., 2015). In the three studies mentioned, droplets of lignin on the biomass surface were observed by SEM. Although, in the present work lignin restructuration was not analyzed, these examples illustrate its sensibility for pretreatment conditions.

In regard to the cellulose contained in IF (Fig. 2**(c)**), the statistical analysis shows that its increase is attributed to the combined effect of soda and temperature, as expressed by the linear coefficients a_1 and a_2 of this response. Iso-response curves analysis indicate that NaOH loading lower than 6% at temperatures below 125 °C does not lead to modification of C content in the extrudate. C fraction increases as the NaOH loading is intensified and can attain more than 60% with temperatures higher than 130 °C and NaOH/SCC higher than 6%. The response surface of C content is inverted from the one of H content. The gain of the cellulose portion in the extrudate results from the extraction of hemicelluloses and lignin. Recovering a substrate with a higher cellulose portion than the untreated SCC proves to be an advantage for future saccharification step.

Additionally, cellulose yield (CY) was calculated from the mass balance of the process. A maximum yield of cellulose obtained for soda loadings between 5–7% NaOH/SCC and temperature of 75–125 °C,

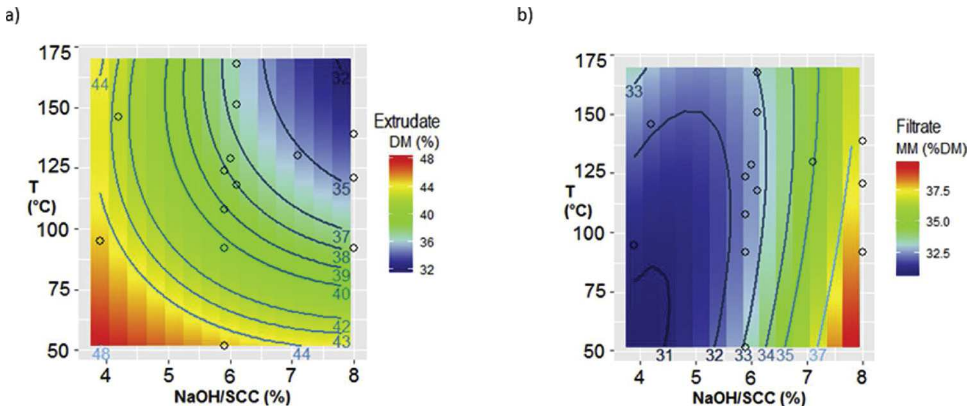

Fig. 3. Iso response curves indicating solid dry matter (DM) of extrudate (a) and mineral matter (MM) of the filtrate (b) as function of the temperature (T) and the NaOH/SCC ratio, the circles represent the experimental results. (To be viewed in color).

reached 90%. Globally, alkali extrusion preserves cellulose through the overall process and variations in different conditions were not significant. However, CY could be affected by the temperature, since at NaOH loading higher than 8% and temperature > 150 °C, the CY decreased. The results obtained imply the possibility of cellulose solubilization or charred as a result of the excessive severity of the pretreatment, as encountered by Silva et al., 2013 when using extrusion with ionic liquid at 180 °C (Silva et al., 2013).

As mentioned earlier, a statistical analysis of the extrudate and filtrate mineral was carried out to study the impact of parameters on filtration efficiency (Fig. 3). The fit is accurate for both responses (Table 4). Concerning extrudate dry matter, the coefficients exhibit that extrudate solid content is modified by the combination of alkali and temperature. It is negatively impacted by increasing reagent input and temperature augmentation (Fig. 3**(a)**). The liquid /solid ratio of base and acid were kept constant in the extrusion process. Therefore, variations of the solid content of the extrudate reflect the filtration performance. Study of IF and H content in extrudate demonstrates the partial lignocellulose solubilization takes place during pretreatment, thereby leading to variations of the biomass viscosity and the dynamic plug stability. Along with the produced IF solubilization, the dynamic plug is softened leading to a decrease in extrudate dry matter.

With respect to the filtrate mineral matter (Fig. 3**(b)**), the coefficients indicate that the soda loading mainly modifies it. As chemicals are added, the content of salts in the filtrate augment, however, as the temperature is increased at high chemical input, there is a decrease of the mineral matter, implying a lower filtration efficiency. Application of this process needs to take into consideration limitations due to the filtration step since higher IF breakdown leads to a weaker dynamic plug. Images of extrudates and filtrates (Figs. S 1 and S 2) depict the impact of the filtration efficiency in extrudate moisture content and solid content of the filtrate. It must be mentioned that filtration is critical in order to ensure the elimination of salts that inhibit fermentation step (Casey et al., 2013; Deparis et al., 2017), and to obtain high extrudate dry matter to easily manage L/S ratios in further phases.

3.2.2. *Enzymatic hydrolysis*

The enzyme efficiency to access polysaccharides for saccharification was mainly evaluated through glucose (GR) and xylose released (XR). These equations express the ability of enzymes to access and produce glucose and xylose from the extrudate. Simultaneously, the process was assessed by the analysis of the glucose and xylose yields. The results from enzymatic hydrolysis after 48 h were summarized in Table 5. Both sugar released and yields were studied statistically. Analysis of the XR was conducted by analysis of coefficients and the iso response curves (Table 6 and Fig. 4), however GR was studied through the values in Table 5 due to poor fitting with the model. General observation showed

Table 5
Enzymatic hydrolysis indicators. Results are based on glucose and xylose released after 48 h, and express the enzymatic performances to digest extrudate (GR and XR) and yield of the overall process (GY and XY).

Sample	NaOH/SCC	Temperature	Enzymatic hydrolysis			
			GR	XR	GY	XY
units	% (w/w)	°C	% g/g cellulose in extrudate	% g/g of hemicelluloses in extrudate	% g/g of dry SCC	% g/g of dry SCC
1	5.9	52	67.7	50.9	23.6	19.4
2	5.9	92	68.6	64.7	23.8	22.5
3	5.9	108	70.3	73.0	24.7	26.7
4	5.9	124	76.0	81.1	25.6	25.8
5	6.1	118	71.3	82.3	25.0	27.6
6	6.0	129	68.3	78.0	25.2	24.5
7	6.1	151	72.4	73.0	23.8	21.0
8	6.1	168	64.4	69.0	23.0	20.4
9	7.1	130	81.0	79.1	27.4	22.8
10	3.9	95	66.8	59.9	21.6	21.8
11	4.2	146	67.5	66.6	23.0	21.9
12	8.0	92	74.9	88.3	24.8	25.5
13	8.0	121	70.9	89.3	22.0	22.4
14	8.0	139	75.4	82.0	22.2	22.2

Note: SCC: Sweet Corn Coproduct; GR: Glucose Released; XR: Xylose Released; GY: Glucose Yield; XY: Xylose Yield.

that the combination of twin-screw extrusion and thermo-chemical action upgraded the action of enzymes on SCC as GC and XC values of extrudates vary from 64% to 81% and 46%–81.7% which is an improvement of by 2–3 times compared to untreated biomass (Table 1). This is associated to the mechanical and chemical actions. The conveying and kneading screws have proven to increase porosity of biomass and reduce of particle size, promoting the enzyme action (Zheng et al., 2015). Using alkaline extrusion, pretreated biomass exposes higher amount of non-crystalline cellulose at the surface, producing higher conversion of carbohydrates than sole alkali pretreated biomass (Khatri et al., 2018).

Increasing the NaOH load clearly leads to a modification of the structure of the lignocellulosic fraction in the extruded matter, primarily by the solvation of hemicelluloses and lignin. Since both components act as a physical barrier enveloping cellulose, its elimination from the substrate can lead to a higher production of glucose by EH. Mild pretreatment conditions lead to less destructuration of lignocellulose, thus lower GC values (Samples 1, 2, 10 and 11). Nonetheless, higher GR can be reached by increasing solubilization of hemicelluloses, either by NaOH increment (Samples 12–14) or by temperature augmentation (Samples 3–5).

Concerning XR, according to the coefficients, the temperature and

Table 6
Statistical analysis of the effect of studied parameters on enzymatic hydrolysis.

	XR		XY		GY	
R^2	0.923		0.762		0.821	
Terms	Estimated	*p* value	Estimated	*p* value	Estimated	*p* value
a_0	76.14	< 0.01***	25.64	< 0.01***	24.95	< 0.01***
a_1	12.041	0.019***	0.84	32.3	0.61	12.2
a_{11}	0.245	92	0.04	96.8	−0.26	53.6
a_2	8.205	0.461**	−2.01	8.3	−2.04	0.238**
a_{22}	−16.789	0.0926***	−6.12	0.328**	−1.56	4.02*
a_{12}	−8.587	9.8	−2.45	24.6	−2.33	3.01*

the NaOH/SCC impact its modification on the conditions studied (Table 6). The linear coefficients a_1 and a_2 are positive, meaning beneficial action of these parameters. However, quadratic coefficient for temperature is negative, putting into evidence a negative influence. As observed in the response surface, the best XR is reached with a combination of NaOH loading beyond 7% and temperature rise ˜125 °C. Although, for all soda concentration, an increase in temperature has an adverse effect. These observations may be attributed to the lignin restructuration. As previously discussed, lignin is profoundly affected by temperature and can undergo structural modifications. In the previous study by Zheng et al., 2015, the action of reverse screw induces high local temperatures that reached transition temperature of lignin. Although it was fixed at 75 °C, the outcome was lignin blocking pores and lower enzyme digestibility. Lignin content is not modified (around 4%) but could undergo structural changes due to temperature and pressure applied by the reverse screw placed after the filtration module (beginning of module 7), provoking the inactivation of enzymatic digestion.

Promoting enzyme efficiency to degrade carbohydrates through optimization of the pretreatment conditions is not the sole objective of this study. High production of fermentable sugar and the deduction of chemicals applied during the pretreatment is crucial to achieving a techno-economical viable process. Consequently, the evaluation of glucose (GY) and xylose yields (XY) is essential to determine the performance of the processes in the studied conditions. Fit for both responses did not reach significant values, in contrast, tendencies can be deduced from statistical analysis (Table 6). Iso-response curves of XY (Fig. 4**(b)**) reveal that for all alkali charges, temperatures between 90 and 125 °C allow the increment of XY beyond 60%. However, temperature and soda can also have adverse effects, for temperature above 150 °C and soda loading higher than 7%. Bringing into comparison the iso-response curves of XR and XY, it shows that XY is not correlated only with enzymatic accessibility. Although high XC is reached with augmented hemicelluloses solubilization, the XY is affected when the hemicelluloses yield decreased by the effect of soda and temperature.

Concerning GY (Fig. 4**(c)**), temperature boost GY between 6–7.5% NaOH/SCC, this effect is also limited for temperatures above 125 °C. This condition is consistent with high values of CY (˜ 90%) and GC (> 70%). These results illustrate the necessity to associate not only high enzymatic digestibility but significant cellulose conservation to achieve high GY. In addition, GY is negatively impacted by temperatures higher than 150 °C and NaOH/SCC ratios beyond 8%, likely due to cellulose deterioration as mentioned earlier. Furthermore, analysis of both iso-response curves allows to conclude that the conditions for optimal glucose and xylose yields are at 6–6.5% NaOH/dry SCC and internal temperature from 90 °C to 125 °C, where GY and XY could reach 25% each (500 g of sugars per 1 Kg of dry SCC).

4. Conclusions

In the present study, the effects of pretreatment alkali loading and temperature in the extruder were investigated. The results underline that several phenomena may be involved in biomass structural modification and the enzymatic hydrolysis. Extrudate insoluble fiber, cellulose and hemicelluloses are mainly affected by the employment of catalyst, and temperature enhances its effect. Under the test conditions, the higher NaOH charge and temperature resulted in the disintegration of hemicelluloses, producing a cellulose-rich substrate. Likewise, it improves the enzymes accessibility resulting from physical barrier elimination. Despite the positive effect of temperature and NaOH/SCC on enzymatic hydrolysis, there are limitations since an adverse effect is observed for cellulose conservation, xylose conversion, and sugar yields. Overall, sugar yield can be improved while decreasing the chemical content in the process through heat.

In terms of process, since there are not competitive uses for this material, the valorization of SCC into bioethanol is economically

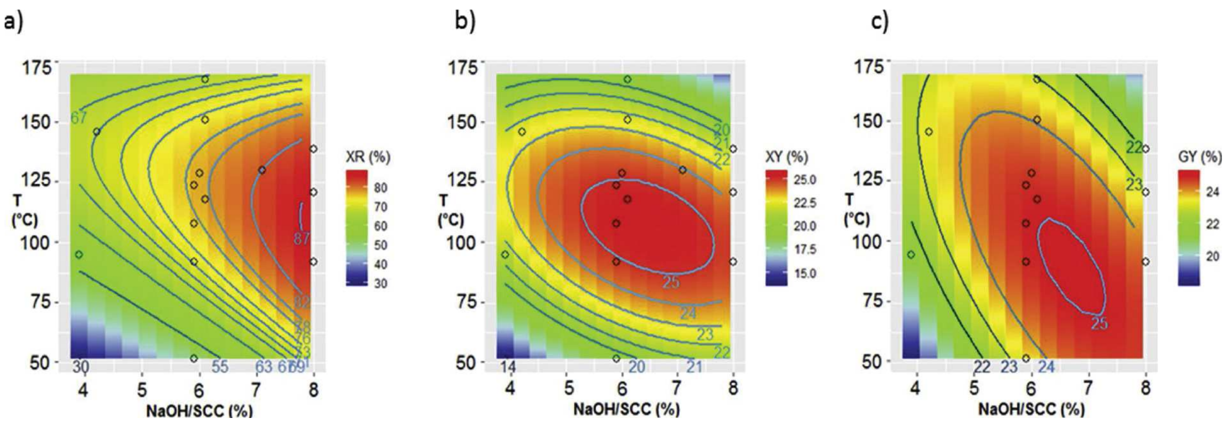

Fig. 4. Iso response curves indicating xylose released rate (%) (% of xylose released/g of hemicelluloses in extrudate)(a), xylose yield (XY)(% g of xylose released/g of dry SCC)(b) and glucose yield (GY)(% g of glucose released/g of dry SCC)(c). The circles represent the experimental results. (To be viewed in color).

interesting for the local market. Considering future steps (impregnation of enzymes, saccharification, and fermentation), the optimal conditions set by this work were at 6–7% NaOH/dry SCC with an internal temperature between 110–130 °C. Compared with conditions employed in earlier works, this study provides a decrease of 3% of sodium hydroxide and 2% of acid inputs. This contributes to the reduction of the pretreatment cost and its environmental impact. Advantageously, in these conditions, filtration is stable and solid content of extrudate is around 36%. This promotes working at high consistency for the future steps and the elimination of inhibitory molecules. The future work will focus on improved enzymatic impregnation for saccharification and co-fermentation at high consistency with additional feasibility studies at pilot scale.

Declarations of interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2019.03.044.

References

Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour. Technol. Special Issue Lignocellulosic Bioethanol. Curr. Status Perspect. 101, 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093.

Casey, E., Mosier, N.S., Adamec, J., Stockdale, Z., Ho, N., Sedlak, M., 2013. Effect of salts on the Co-fermentation of glucose and xylose by a genetically engineered strain of Saccharomyces cerevisiae. Biotechnol. Biofuels 6 (83). https://doi.org/10.1186/1754-6834-6-83.

Chen, Y., Stevens, M.A., Zhu, Y., Holmes, J., Xu, H., 2013. Understanding of Alkaline Pretreatment Parameters for Corn Stover Enzymatic Saccharification. pp. 10.

Cherubini, F., Ulgiati, S., 2010. Crop residues as raw materials for biorefinery systems – a LCA case study. Appl. Energy 87, 47–57. https://doi.org/10.1016/j.apenergy.2009.08.024.

Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P., Cherchi, F., 2012. Review of Pretreatment Processes for Lignocellulosic Ethanol Production, and Development of an Innovative Method. Biomass and Bioenergy, International Conference on Lignocellulosic Ethanol 46, 25–35. https://doi.org/10.1016/j.biombioe.2012.04.020.

Deparis, Q., Claes, A., Foulquié-Moreno, M.R., Thevelein, J.M., 2017. Engineering tolerance to industrially relevant stress factors in yeast cell factories. FEMS Yeast Res. 17. https://doi.org/10.1093/femsyr/fox036.

Donohoe, B.S., Decker, S.R., Tucker, M.P., Himmel, M.E., Vinzant, T.B., 2008. Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment. Biotechnol. Bioeng. 101, 913–925. https://doi.org/10.1002/bit.21959.

Duque, A., Manzanares, P., Ballesteros, I., Negro, M.J., Oliva, J.M., Saez, F., Ballesteros, M., 2013. Optimization of integrated alkaline–extrusion pretreatment of barley straw for sugar production by enzymatic hydrolysis. Process. Biochem. 48, 775–781. https://doi.org/10.1016/j.procbio.2013.03.003.

Duque, A., Manzanares, P., Ballesteros, M., 2017. Extrusion as a pretreatment for lignocellulosic biomass: fundamentals and applications. Renew. Energy 114, 1427–1441. https://doi.org/10.1016/j.renene.2017.06.050.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science 327, 812–818. https://doi.org/10.1126/science.1185383.

IRENA, 2016. Innovation Outlook Advanced Biofuels 2016 Summary | Biofuel | Gasification.Innovation Outlook Advanced Biofuels 2016 Summary | Biofuel | Gasification.

Kaar, W.E., Holtzapple, M.T., 2000. Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover. Biomass Bioenergy 18, 189–199. https://doi.org/10.1016/S0961-9534(99)00091-4.

Khatri, V., Meddeb-Mouelhi, F., Adjallé, K., Barnabé, S., Beauregard, M., 2018. Determination of optimal biomass pretreatment strategies for biofuel production: investigation of relationships between surface-exposed polysaccharides and their enzymatic conversion using carbohydrate-binding modules. Biotechnol. Biofuels 11. https://doi.org/10.1186/s13068-018-1145-5.

Kim, J.S., Lee, Y.Y., Kim, T.H., 2016. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour. Technol. Pretreat. Biomass 199, 42–48. https://doi.org/10.1016/j.biortech.2015.08.085.

Leggett, L.M.W., Ball, D.A., 2012. The implication for climate change and peak fossil fuel of the continuation of the current trend in wind and solar energy production. Energy Policy 41, 610–617. https://doi.org/10.1016/j.enpol.2011.11.022.

Liu, C., van der Heide, E., Wang, H., Li, B., Yu, G., Mu, X., 2013. Alkaline twin-screw extrusion pretreatment for fermentable sugar production. Biotechnol. Biofuels 6 (97). https://doi.org/10.1186/1754-6834-6-97.

Liu, H., Pang, B., Zhao, Y., Lu, J., Han, Y., Wang, H., 2018. Comparative study of two different alkali-mechanical pretreatments of corn stover for bioethanol production. Fuel 221, 21–27. https://doi.org/10.1016/j.fuel.2018.02.088.

Modenbach, A., 2013. Sodium Hydroxide Pretreatment of Corn Stover and Subsequent Enzymatic Hydrolysis: an Investigation of Yields, Kinetic Modeling and Glucose Recovery. Theses and Dissertations–Biosystems and Agricultural Engineering.

Prior, B.A., Day, D.F., 2008. Hydrolysis of ammonia-pretreated sugar cane bagasse with cellulase, β-Glucosidase, and hemicellulase preparations. Appl. Biochem. Biotechnol. 146, 151–164. https://doi.org/10.1007/s12010-007-8084-0.

Putro, J.N., Soetaredjo, F.E., Lin, S.-Y., Ju, Y.-H., Ismadji, S., 2016. Pretreatment and conversion of lignocellulose biomass into valuable chemicals. RSC Adv. 6, 46834–46852. https://doi.org/10.1039/C6RA09851G.

Rodrigues, C.I.S., Jackson, J.J., Montross, M.D., 2016. A molar basis comparison of calcium hydroxide, sodium hydroxide, and potassium hydroxide on the pretreatment of switchgrass and miscanthus under high solids conditions. Ind. Crop. Prod. 92, 165–173. https://doi.org/10.1016/j.indcrop.2016.08.010.

Saha, B.C., 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30, 279–291. https://doi.org/10.1007/s10295-003-0049-x.

Sannigrahi, P., Kim, D.H., Jung, S., Ragauskas, A., 2011. Pseudo-lignin and pretreatment chemistry. Energy Environ. Sci. 4, 1306–1310. https://doi.org/10.1039/C0EE00378F.

Schleussner, C.-F., Rogelj, J., Schaeffer, M., Lissner, T., Licker, R., Fischer, E.M., Knutti, R., Levermann, A., Frieler, K., Hare, W., 2016. Science and policy characteristics of the Paris Agreement temperature goal. Nat. Clim. Change 6, 827–835. https://doi.org/10.1038/nclimate3096.

Sharma, R., Palled, V., Sharma-Shivappa, R.R., Osborne, J., 2013. Potential of potassium hydroxide pretreatment of switchgrass for fermentable sugar production. Appl. Biochem. Biotechnol. 169, 761–772. https://doi.org/10.1007/s12010-012-0009-x.

Silva, A.S., da, Teixeira, R.S.S., Endo, T., Bon, E.P.S., Lee, S.-H., 2013. Continuous pretreatment of sugarcane bagasse at high loading in an ionic liquid using a twin-screw extruder. Green Chem. 15, 1991–2001. https://doi.org/10.1039/C3GC40352A.

Soest, V.J.P., Wine, R.H., 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. J. Assoc. Off. Anal. Chem. 51, 780–785.

Solomon, B.D., 2010. Biofuels and sustainability. In: Limburg, K., Costanza, R. (Eds.), Ecological Economics Reviews. Wiley-Blackwell, Malden, pp. 119–134.

Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol. Rev. Issue 83, 1–11. https://doi.org/10.1016/S0960-8524(01)00212-7.

Vandenbossche, V., Doumeng, C., Rigal, L., 2014. Thermomechanical and thermo-mechano-chemical pretreatment of wheat straw using a twin-screw extruder. BioResources 9, 1519. https://doi.org/10.15376/biores.9.1.1519-1538. 1538–1538.

Vandenbossche, V., Brault, J., Vilarem, G., Rigal, L., 2015. Bio-catalytic action of twin-screw extruder enzymatic hydrolysis on the deconstruction of annual plant material: case of sweet corn co-products. Ind. Crops Prod. 67, 239–248. https://doi.org/10.1016/j.indcrop.2015.01.041.

Vandenbossche, V., Brault, J., Hernandez-Melendez, O., Evon, P., Barzana, E., Vilarem, G., Rigal, L., 2016. Suitability assessment of a continuous process combining thermo-mechano-chemical and bio-catalytic action in a single pilot-scale twin-screw extruder for six different biomass sources. Bioresour. Technol. 211, 146–153. https://doi.org/10.1016/j.biortech.2016.03.072.

Zabed, H., Sahu, J.N., Boyce, A.N., Faruq, G., 2016. Fuel ethanol production from lignocellulosic biomass: an overview on feedstocks and technological approaches. Renew. Sustain. Energy Rev. 66, 751–774. https://doi.org/10.1016/j.rser.2016.08.038.

Zabed, H., Sahu, J.N., Suely, A., Boyce, A.N., Faruq, G., 2017. Bioethanol production from renewable sources: current perspectives and technological progress. Renew. Sustain. Energy Rev. 71, 475–501. https://doi.org/10.1016/j.rser.2016.12.076.

Zhang, X., Xu, J., Cheng, J.J., 2011. Pretreatment of corn stover for sugar production with combined alkaline reagents. Energy Fuels 25, 4796–4802. https://doi.org/10.1021/ef201130d.

Zheng, J., Choo, K., Rehmann, L., 2015. The effects of screw elements on enzymatic digestibility of corncobs after pretreatment in a twin-screw extruder. Biomass Bioenergy 74, 224–232. https://doi.org/10.1016/j.biombioe.2015.01.022.

1. Supplemental files

Table S 1: Normalized data using Z-score method

Experimental design				
Sample	NaOH/SCC	T	X1	X2
units	% (w/w)	°C		
1	5.9%	52	-0.0500	-1.0000
2	5.9%	92	-0.0500	-0.3103
3	5.9%	108	-0.0500	-0.0345
4	5.9%	124	-0.0500	0.2414
5	6.1%	118	0.0500	0.1379
6	6.0%	129	0.0000	0.3276
7	6.1%	151	0.0500	0.7069
8	6.1%	168	0.0500	1.0000
9	7.1%	130	0.5500	0.3448
10	3.9%	95	-1.0500	-0.2586
11	4.2%	146	-0.9000	0.6207
12	8.0%	92	1.0000	-0.3103
13	8.0%	121	1.0000	0.1897
14	8.0%	139	1.0000	0.5000

SCC: Sweet corn co-product; T: temperature probe 1

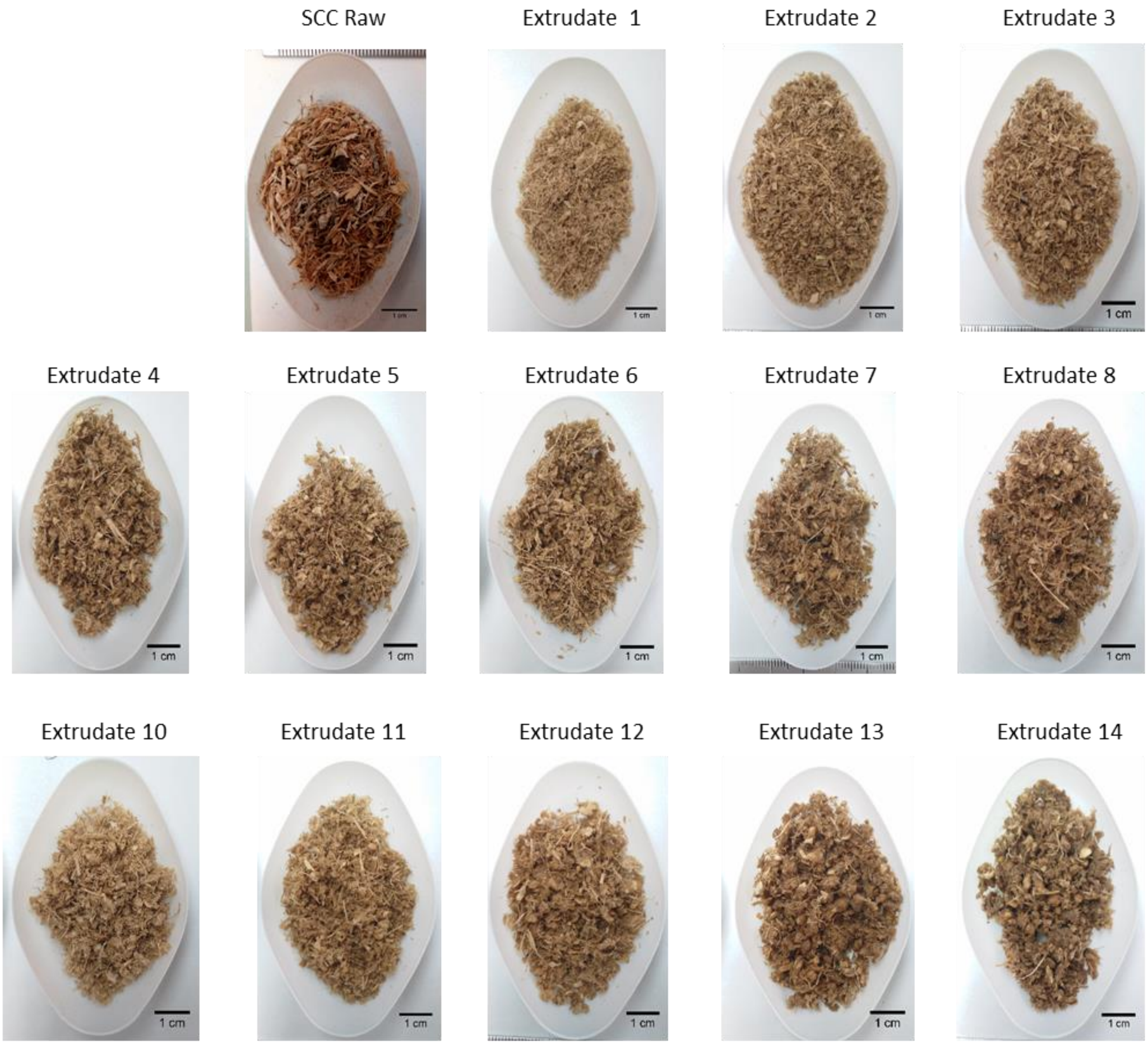

Figure S 1: Photos of untreated biomass and extrudates obtained with part conditions evaluated in the present work

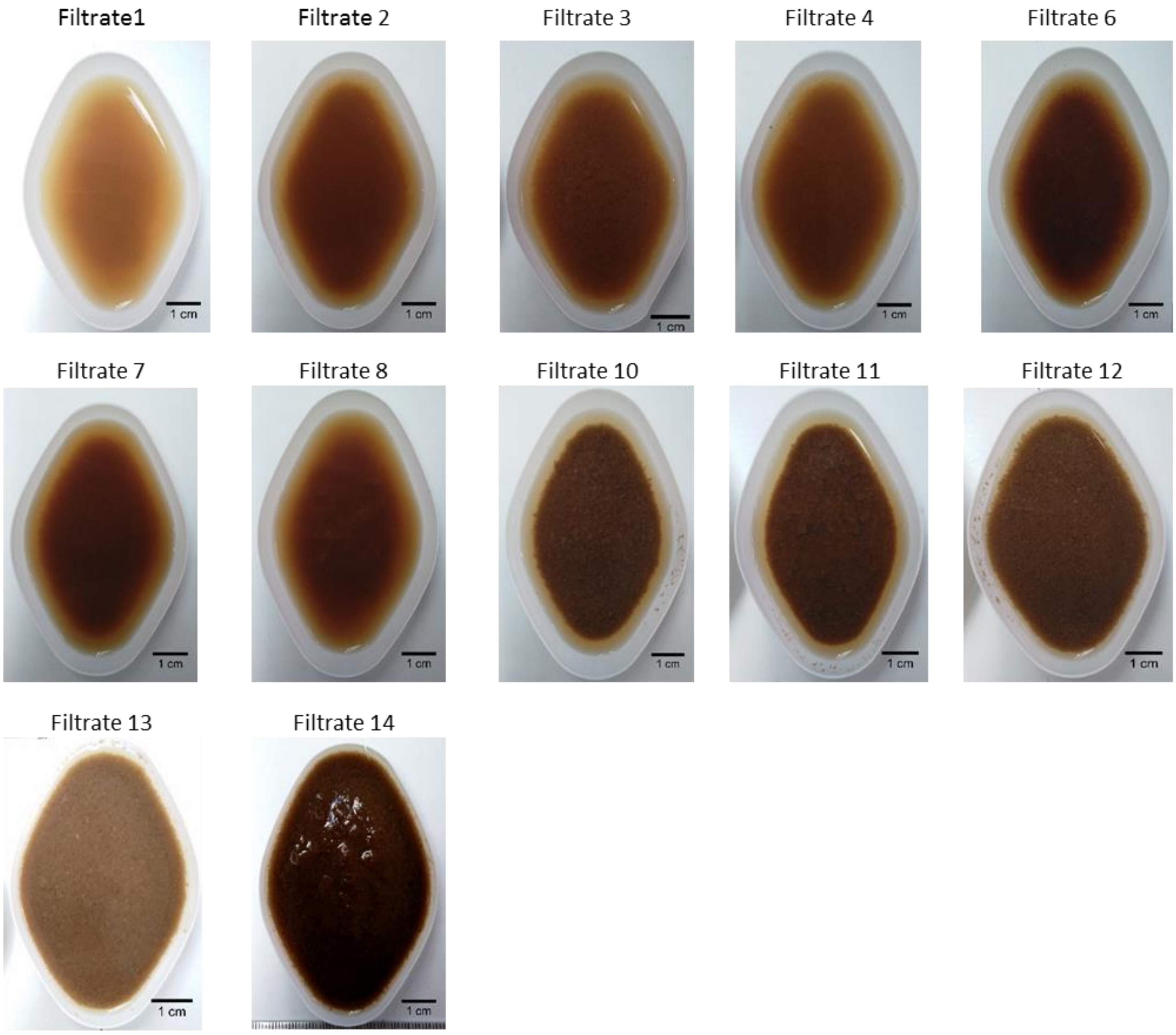

Figure S 2: Photos of filtrates obtained with part of the conditions evaluated in the present work