Enhanced enzymatic hydrolysis of corn stover using twin-screw extrusion under mild conditions

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Abstract

This paper aims to investigate the effect of extrusion at high solid loading on corn stover (CS) properties and its enzymatic hydrolysis. This biomass was extruded under different screw speeds and different solid loadings and the impact of these parameters on physicochemical properties was evaluated. It was found that lignocellulosic components were not significantly affected by the pretreatment, while surface area increased with solid loading and rotation speed. Different enzyme cocktails were used for the enzymatic hydrolysis of extruded and untreated CS. Overall, mild twin-extrusion enhanced the enzymatic hydrolysis of corn stover through an increase in glucose and xylose yields by 134-212% and 214-294% respectively when using *T. longibrachiatum* cellulase. The highest sugar content was obtained from CS extruded under 400 g total solids (TS) per liter and 200 rpm. The energy efficiency of the pretreatment was also assessed and was found to be maximal at 400 gTS/L and 200 rpm.

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Keywords: Biomass, Pretreatment, Biorefinery, Extrusion, Enzymes, Biofuel

Abbreviations

CrI crystallinity index;

CS corn stover;

XRD X-ray diffraction;

FPU filter-paper units;

SME specific mechanical energy;

TSE twin-screw extrusion;

TS total solids

1. Introduction

Bioconversion of residual lignocellulosic biomass to bioethanol has shown these last decades environmental, economic and energetic advantages in comparison to bioethanol produced from starch or sugar derived from sugar cane and maize (1). Lignocellulosic biomass is an abundant, cheap, and renewable organic resource with a heterogeneous structure mainly composed of cellulose, hemicelluloses, and lignin. After hydrolysis of cellulose and hemicellulose, the carbohydrate components (glucose and xylose) are fermentable, which makes lignocellulosic biomass an appropriate feedstock for bioenergy and biofuel production (2). Nevertheless, the structural and chemical properties of lignocellulosic biomass, make it extremely resistant to biodegradation by enzymes and microbial attacks (3).

In addition, enzymes can be used to biologically degrade the lignocellulose for bioethanol production. These enzymes can be produced in-situ by micro-organisms (4). Micro-organisms, mainly white and soft-rot fungi, actinomycetes, and bacteria can be used to pretreat lignocellulosic wastes. They can solubilize lignin due to the action of enzymes such as peroxidases and laccases. However, the effectiveness of enzymes can be improved when breaking down the lignocellulosic structure first (5). In general, a suitable pretreatment procedure involves (i) cleaving cross-linked matrix in hemicelluloses and lignin, (ii) increasing the porosity and surface area of cellulose for subsequent enzymatic hydrolysis, and finally (iii) disrupting hydrogen bonds in crystalline cellulose (5).

Various pretreatment approaches including physical, chemical, physicochemical, and biological methods have been developed to improve the enzymatic saccharification of biomass (6).

For instance, enzymatic hydrolysis of wheat straw was enhanced after hot water pretreatment under 180°C for 10 min (7). Moreover, the addition of dilute acid (1% of H_2SO_4, 1 h at 220°C) to steam explosion pretreatment increased arabinose and xylose content by 200% and 61% compared to untreated barley straw (7). Acid pretreatment can lead to high solubilization of hemicelluloses and cellulose, but at high temperatures and high acid concentration, inhibitory compounds (furfural, 5-HMF) can be generated (8). Furthermore, alkaline pretreatment favors lignin degradation and alteration, while increasing the accessible surface area (9). Ammonia fiber explosion has been shown to increase pore size in corn stover leading to a higher ethanol production compared to dilute acid pretreatment (10). Biological pretreatments using fungus can also be applied to lignocellulosic biomasses for delignification to enhance enzymatic hydrolysis (11). However, these methods require the use of acids, bases, salts, solvents, and micro-organisms, which introduce additional costs (12) and may have a negative environmental impact. Ultrasonic and microwave pretreatments were found to enhance sugar solubilization and their efficiency depended on the energy supplied. In the last decade, the extrusion process has been widely used in the food, feed, and plastic industries. As a result, extrusion has been identified as an interesting alternative method for fractionation of lignocellulosic biomass based on its ability to provide high shear, rapid heat transfer, and effective and fast mixing (13). It is a highly versatile pretreatment that can be used alone as a physical pretreatment (14,15) or combined with chemicals and other pretreatment methods (16,17). In addition, as a continuous pretreatment process, extrusion has a higher processing capacity when compared to batch systems (18). Furthermore, extrusion pretreatment has the advantage of avoiding the generation of inhibitors, except when the extrusion is carried out in combination with chemical pretreatments (19). Furthermore, compared to traditional physical methods such as chopping, grinding, and milling, extrusion not only reduces the particle size but also provides a thermochemical pretreatment (20,21). The extrusion process has many advantages like the ability to provide high shear, rapid heat transfer, and effective mixing (21), which leads to an increase in the surface area of the cellulose available to the enzymes (12). Structural changes are mainly promoted in the mixing zones of the screws formed by the successive kneading elements connected with a slight deviation angle between them, which depends on the extrusion purpose.

Previous studies have shown the interest of extrusion pretreatment on enzymatic hydrolysis of biomass. Myat and Ryu (2014) obtained 28% higher ethanol production due to twin-screw extrusion (22), while Han et al. (2013) and Kang et al. (2013) reported that, around 20% and 25% of solid to liquid ratio, ethanol content was at its maximal value (23,24). Although extrusion had a positive impact on sugar release, the effect of operating conditions was not significant in contrast to enzyme source and dose (18).

Indeed, several studies have been conducted on the effect of extrusion operating conditions. However, the evaluation of the energy-based efficiency is missing in these studies. In addition, most of the studies are carried out at high temperatures, as well as in the presence of chemical reagents. Therefore, the present study assesses the effect of extrusion on the enzymatic hydrolysis under mild conditions ensuring environmentally friendly solubilization of corn stover. The effect of two different enzymes cocktails on untreated and pretreated corn stover hydrolysis was investigated. The physicochemical properties changes, enzymatic digestibility, energy consumption, and efficiency were evaluated and compared to select the suitable conditions for the enzymatic hydrolysis of pretreated corn stover.

2. Materials and Methods

2.1. Biomass preparation

Corn stover was obtained from (the Occitanie department, Toulouse, France). Before pretreatment, CS was chopped to a particle size of approximately 10-20 cm using a vegetable-milling machine. The chopped samples were then ground using knife milling (Retsch SM 100, Germany) with a screen size of 4 mm. The substrate was dried and stored at room temperature. Raw CS contained 37.5% (TS basis - w/w) of cellulose, 22.3% (TS basis) of hemicelluloses, and 25.2% (TS basis) of lignin. In addition, carbon and oxygen contents were 46% and 48% (TS basis) respectively. Therefore, CS can be considered as a biomass of interest for conversion into biofuels (25).

2.2. Continuous pretreatment using a twin-screw extruder

The extrusion pretreatment was conducted using a twin-screw extruder (Eurolab 16, Thermo Scientific, USA). The barrel had a diameter of 16 mm and the length to diameter ratio (L/D) of the screws was 25. The experimental setup used in this study is illustrated in **Fig. 1**.

Insert Figure 1

The extruder contains three different elements: forward screw elements, which enable the continuous movement of the material from inlet to outlet; kneading screw elements, which exert an effective mixing and shearing effect on the biomass; and reverse screw elements, which move the material backward and increase its residence time to improve the pulverization effect (19).

Table 1 presents the operating conditions used in this study. The ground biomass was impregnated with water using a spray system to reach the different solid/liquid ratios. To reduce the energy demand of the pretreatment, the temperature was maintained at 37°C and the two parameters considered were extrusion speed and solid load. However, it should be noted that other factors are also important such as extruder design, reagent addition, feed rate, and also the size of the biomass at the process inlet. It was chosen to vary only the rotation speed and the solid/liquid ratio which, in addition to the temperature, are reported as the most influencing parameters (26).

2.3. Enzymatic hydrolysis of CS biomass

The efficiency of extrusion for the CS pretreatment was evaluated through the enzymatic hydrolysis using different enzyme cocktails. Cellulose and hemicelluloses, which represent two-thirds of cell wall dry matter, can be degraded into sugars. For this purpose, the enzymatic hydrolysis of untreated and pretreated CS by different enzymatic cocktails was evaluated. Cellulase from T. *longibrachiatum* ≥ 1mg/unit (C9748), and T. *reesei* (C2730) were obtained from Sigma Aldrich and were added under the same conditions (with a dose of 30 U/g TS). Enzymatic hydrolysis was carried out at a solid concentration of 5% (w/v) in a 50 mM sodium acetate buffer (pH 5.0) at 37 °C for 72 h with agitation (100 rpm). After enzymatic hydrolysis, the supernatant was filtered (0.45 µm) and the enzymatic digestibility was assessed by the amount of sugar released (i.e. glucose, xylose, arabinose in mg g^{-1} CS) determined according to the procedure described previously (27). The sugars contained in the enzymatic hydrolysates were analyzed by high-performance liquid chromatography (HPLC) using the BioRad HPX-87P and HPX87H columns (28). All the experiments were operated in triplicate, and the average value was reported.

The results were compared in pairs using the Student's t-test, assuming equal variance, normality, and independence of repetitions.

2.4.Biochemical and structural analysis of substrates

The carbohydrate and lignin concentrations in lignocellulose samples (untreated and pretreated CS) were determined using the Klason method described in (28) and (29). All the values reported here were duplicate results. The crystallinity of untreated and pretreated CS was measured using an X-ray diffractometer (XRD, Bruker diffractometer D8 Advance). The measurements were carried out on compacted powder on small mats. X-ray diffraction (XRD) data were collected from 2θ = 5° to 50° with a step interval of 0.02°. The crystallinity index (CrI) was defined as follows (**Eq.1**):

$$\boldsymbol{CrI = (I_{002} - I_{am})/I_{002} \times 100} \qquad \text{(Eq.1)}$$

Where I_{002} and I_{am} are the peak intensities of crystalline and amorphous cellulose, respectively.

Particle size was analyzed by laser granulometric (Mastersizer 2000, Malvern Instrument, USA) and the surface area of untreated and pretreated CS samples was determined using the BET (Micromeritics, ASAP2020, USA) method. The gas adsorption data was collected using a Micro-metrics (France) 3Flex Surface characterization analyzer using N_2. Before N_2 sorption, the samples were degassed at 50°C overnight. The specific surface areas were determined from the nitrogen adsorption/desorption isotherms (at -196°C). The swelling capacity of the powdered samples was measured using the bed volume technique (30). 100 mg of sample was dispersed in 10 mL of distilled water and left overnight at room temperature. The volume of the swollen sample was then measured and expressed as mL of swollen sample per g of original dry sample (30). The conversion yields of cellulose and xylose after enzymatic hydrolysis are calculated using the following equations (**Eq.2** and **Eq.3**):

$$Glucose\ yield\ (\%) = \frac{Glucose\ in\ enzymatic\ hydrolysate (g/100gTS)}{Cellulose\ in\ biomass\ (pretreated\ or\ not)(g/100gTS)} x100\ (Eq.2)$$

$$Xylose\ yield\ (\%) = \frac{Xylose\ in\ enzymatic\ hydrolysate (g/100gTS)}{Hemicelluloses\ in\ biomass\ (pretreated\ or\ not)(g/100gTS)} x100\ (Eq.3)$$

After the extrusion, the liquid and solid fractions were separated by filtration. The liquid was then analyzed to assess the solubilization effect of extrusion by determining sugar content using the HPLC.

2.5. Specific energy consumption and pretreatment efficiency

To evaluate the energy efficiency of the extrusion pretreatment, the electric energy consumed by the extruder was determined based on the torque factor. Torque indicates the power consumption of the electric motor while specific mechanical energy (SME) is the amount of power that is being input by the motor into each kilogram of material being processed (kWh kg^{-1} TS). Torque (τ) was measured directly by the extruder system (Nm). The total energy requirement (SME) of different extrusion experiments was calculated using **Eq.4** (13):

$$SME = \frac{\tau * 2\pi * \text{speed} * 60}{m} \qquad (Eq.4)$$

Torque and SME of different extrusion pretreatments depend on the physicochemical properties of biomass and also on the initial particle size, which also depends strongly on the extrusion parameters and conditions (speed, screw profile, solid loading, temperature…).

The efficiency of the pretreatment was given by the following equation **(Eq.5):**

$$\square = \frac{Sugars\ yield(or\ Glucose)\ (g/kg\ CS)}{SME\ (kWh/kg\ CS)} \qquad (Eq.5)$$

3. Results and discussion

3.1. Effect of twin-screw extrusion on CS properties and composition

Table 2 presents the main physicochemical properties and composition of raw and pretreated CS under different extrusion conditions. Extrusion increased surface area and reduced CS swelling. In addition, the soluble sugar content was not significantly increased after extrusion. However, in Huang et al. (2016) swelling property of orange pomace was increased by 85% after extrusion under 125°C, 290 rpm, and 14% of moisture (31). In this study, any modification of the CS fiber compositions may be due to the lignocellulosic CS complex matrix and to the low applied temperature of 37°C compared to 125°C (31). Furthermore, an increase in solid loading and speed of extruder was advantageous in reducing the particle size of pretreated CS, except for extrusion under 400 rpm and 300 gTS/L, which may be explained by CS lignocellulosic matrix heterogeneity. The particle size of raw CS was around 4 mm and decreased to 0.42 mm when the sample's loading achieved 600 gTS /L at a rotation screw speed of 300 rpm. In general, the available surface area is closely related to the particle size and the volume of the pores generated by the pretreatment. Extrusion has been reported to cause physical disruption of cell wall assembly due to the combination of thermal and mechanical energy (shearing forces), leading to a partial deconstruction of biomass structure. In addition, extrusion reduced the crystallinity of CS from 46 to 30 %.

Table 3 presents the impact of extrusion on the solubilization of different biomasses reported in the literature. Previously, Aktas-Akyildiz et al. (2020) reported that the extrusion speed affected the solubilization of sugars in wheat bran. Under the same temperature and solid loading conditions, the soluble sugar content achieved the double, when the screw speed was increased from 100 to 200 rpm (32). In the same manner, Zhang et al. (2012) found that glucose yield from CS increased with screw speed up to 100 rpm when using two temperature zones (50-140°C). However, above 100 rpm, the glucose yield was decreased. Moreover, Zhang et al. 2012 reported that, below 100 rpm, moisture content affected positively the glucose and xylose yields (33). In contrast, Haghighi-Manesh and Azizi (2018) reported that moisture above 50% reduced the solubilization of sugars (34). However, this negative effect of moisture was not observed in our study, which may be due to the low temperature used. Zhang et al. (2020) studied the effect of extrusion operating parameters on the degree of expansion that is related to the release of sugars from the rice husk. Biomass size, moisture, temperature, and screw speed can highly impact the solubilization of sugars (35). Based on sugar release yields, the optimum screw speed and biomass humidity at temperatures around 60°C will not be the same as at high temperatures (> 100°C). However, from an economic point of view, low-temperature pretreatment is more advantageous if the other parameters are well optimized. An increase of glucose of 38% was obtained from corn starch after extrusion under 120 rpm with a moisture of 33% at 70°C (36). Although, Zeng et al. (2017) did not report any solubilization improvement compared to control when pretreating brown rice at 60°C, 100 rpm, and 30% of humidity (37). This finding was in accord with the results of this study. To increase the efficiency of the extrusion, chemicals can be added which enhance the degradation of lignocellulose and thus, improve soluble sugar content (38–40). Extrusion can also be followed by ultrasonic for further solubilization of biomass fibers (35).

3.2. Enzymatic hydrolysis of untreated and pretreated CS

The main effects of screw speed and solid load (**Table 1**) were evaluated using different enzymes by determining the total sugars, glucose, and xylose released after hydrolysis as illustrated in **Fig.2**. The release of glucose and xylose by T. *reesei* or T. *longibrachiatum* cellulases was enhanced by extrusion pretreatment. Extrusion enhanced glucose release by 21-48 % for *T. reesei* cellulase while *T. longibranchiatum* enabled an increase of 139-209% due to extrusion (**Fig.2b**). Xylose content in the hydrolysate was increased by 20-59% and 220-286 % when using *T. reesei* and *T. longibranchiatum* respectively. Therefore, extrusion had a positive effect on enzymatic hydrolysis. Even if extrusion had no drastic change in biomass composition, it resulted in particle size decrease, morphology, and surface aspect changes (41). The biomass structure became more accessible for the enzymes therefore easier to hydrolyze which improved the sugars yield. Glucose and xylose yields were also reported in **Fig.2**. It can be shown that for all conditions, extrusion enhanced cellulose and hemicelluloses conversion into glucose and xylose. The highest glucose yields were achieved with cellulase from *T. longibrachiatum* with up to 200 % improvement over untreated CS. Overall, the glucose conversion yield remained below 36% which was quite low compared to wood chips (45%) (42) and banana peel (65%) (43). Thus, the conversion yield depends on biomass and enzymatic hydrolysis conditions.

Moreover, according to **Fig.2**, cellobiose release was obtained using cellulase from *T. reesei* cocktail. Indeed, *T. reesei* cellulase is composed of two cellobiohydrolases (44) and low β-glucosidase activity which explains the release of cellobiose. On the other hand, *T. longibrachiatum* contains different xylanase and accessory activities (45) which may explain the high effectiveness of this cocktail. In addition, there is a slight difference between the molecular weight of these enzyme cocktails (68 kDa and 57.250 kDa for T. *reesei* and T. *longibrachiatum* cellulases respectively) which may affect the hydrolysis yield (46). However, no difference was noticed between the results obtained from T. *reesei* and T. *longibrachiatum* cellulases on the untreated CS. This can be explained by a reduction of the effect of T. *longibrachiatum* on the control due to untreated CS size, which decreased the contact surface. In addition, it should be noticed that, in the case of enzymatic hydrolysis with *T. reesei* cocktail, the best sugar solubilization was observed for the extrudate sample with 600 gTS /L of solid load and at a rotation screw speed of 300 rpm. In these operating conditions, the extrudate CS presented the highest surface area (about 1.697 m^2 g^{-1}) which improved the contact surface of the enzymes with the substrate during conversion (47). Regarding the cocktail from *T. longibrachiatum,* the best sugar release was obtained after the extrusion of CS at 400 g/L and 200 rpm. As this enzyme source was the most effective in sugar hydrolysis, it was retained for energy efficiency calculations. As seen previously, extrusion can lead to physical changes in biomass matrix and cellulose crystallinity, which allows for enhanced accessibility and thus, improved sugar release after enzymatic hydrolysis. Particle size was found to highly affect the hydrolysis rate (48). Kapoor et al. 2019 reported that a reduction of rice straw size from 20 to 5 mm increased glucan solubilization from 8.5 to 17.7% (49). Cellulose crystallinity and lignin content were also reported as key factors of enzymatic hydrolysis efficiency (50). The crystalline cellulose is characterized by its high density, strong intramolecular bonding, and compact arrangement. The reduction of crystallinity was found to be significantly related to enzymatic hydrolysis efficiency (51). In addition, a high lignin content can inhibit enzymatic hydrolysis as cellulase can be adsorbed onto lignin and thus can be deactivated (52). However, in the case of this study, enzymatic hydrolysis of extruded corn stover resulted in higher soluble sugars even if fiber content was not affected by extrusion. Myat and Ryu (2014) reported an enhancement of 20% of xylose after enzymatic hydrolysis, due to extrusion under 140°C with 0.75% NaOH added (22). The operating conditions of extrusion can affect the enzymatic hydrolysis results as shown by (23,53).

The screw speed was found to positively affect the enzymatic hydrolysis efficiency through glucose release enhancement (53), while Han et al. (2013) reported a negative impact of temperature increase in glucose content after enzymatic hydrolysis (23). Extrusion pretreatment can be combined with acid or alkaline reagents addition under relatively high temperatures, promoting thus, the solubilization of biomass (18,24). However, the energy consumption of the process must be taken into account, as well as the profitability of the recovery of ethanol after fermentation (23).

Insert Figure 2

For efficient enzymatic hydrolysis, a synergistic enzyme combination is recommended (44). **Fig. 3** shows the amount of released sugars from *T. longibrachiatum* cellulose completed with xylanase. For both untreated CS and CS (400/200), the addition of xylanase was effective in enhancing the release of xylose and glucose. Indeed, xylanase hydrolyzes the xylan bound around the cellulose and therefore the cellulose becomes more accessible to the cellulase, whereas the cellulase allows better accessibility of the xylan. Thus, it has been shown that there is a real synergistic effect between xylanase and cellulase (54). Overall, the highest sugar yield was obtained from CS (400/200) using *T. longibrachiatum* cellulase in synergy with xylanase which released both glucose and xylose.

Insert Figure 3

3.3. Pretreatment efficiency

Fig.4 presents the effect of extrusion conditions on torque and specific energy requirement. Interestingly, the torque decreased when screw speed and moisture content increased, in accord with (32). Torque decreased also when temperature increased (13). Moreover, torque is mainly affected by screw speed, degree of filling of the barrel, and viscosity of the biomass (55). Chen et al. (2011) reported torque values ranging from 107 Nm (150 rpm at 500 g/L) to 202 Nm (30 rpm at 500 g/L) during twin-screw extrusion of rice straw (56). As temperature and moisture impact the viscosity, torque is therefore affected. Mass flow and particle size are also affecting the torque. Lignocellulosic biomass nature can also modify the torque. Karunanithy and Muthukumarappan (2010) reported that torque was 100 Nm and 70 Nm for switchgrass and corn stover (155 rpm and 500 g/L), respectively (14). Overall, among the extruder conditions, the temperature was the most influencing parameter both on torque and specific mechanical energy (SME) (55). In the present study, specific mechanical energy was not significantly affected by either the screw speed or the moisture content of CS, suggesting that the pretreatment efficiency of each experiment will depend mainly on sugar release.

Insert Figure 4

Fig.5 shows the impact of extrusion conditions on pretreatment energy efficiency. Pretreatments under 400 gTS/L and 200 rpm and 600 gTS/L and 100 rpm resulted in the highest energy efficiency. It should be pointed out that these results are only relevant at 37°C. At high temperatures, thermal energy should be added to the specific mechanical energy to calculate the pretreatment efficiency. To enhance the energy recovery from corn stover, anaerobic digestion of the solid residue can be considered after enzymatic hydrolysis (40).

Although sugar yields are still lower (27-36%) than what can be obtained by chemical pretreatment (up to 90%) (57), this yield is higher than that obtained after ball milling, with a glucose yield of 25% for corn stover (58). The most important advantage of extrusion is the use of a high solid load and no chemical reagent was added. This suggests that liquid pretreatment management costs should not be assumed contrary to the case of chemical pretreatment. It should be noted that the cost of treating a pretreatment liquid is around 20% of the bioethanol production unit costs (59,60).

Insert Figure 5

4. Conclusions

In this study, an innovative treatment using twin-extrusion without chemical addition was studied at low temperature and with high solid loading. No effect was observed on chemical and biochemical composition, however, extrusion affected significantly the physical structure of CS biomass while increasing cellulose and xylan enzymatic accessibility. Although the sugar yield obtained in this work was low compared to severe pretreatments, the use of mild conditions saves energy while not adversely affecting the environment. The highest sugar content was obtained from CS extruded under 400 gTS/L and 200 rpm. The energy efficiency of the pretreatment was also assessed and was found to be maximal at 400 gTS/L and 200 rpm. Twin-screw extrusion was a suitable pretreatment prior to enzymatic hydrolysis of biomass in particular corn stover. Therefore, twin-extrusion can be considered as a clean way to pretreat the biomass and therefore enhance subsequent sugar release and thus ethanol production.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Haghighi Mood S, Hossein Golfeshan A, Tabatabaei M, Salehi Jouzani G, Najafi GH, Gholami M, et al. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renewable and Sustainable Energy Reviews. nov 2013;27:77-93.

2. Vasić K, Knez Ž, Leitgeb M. Bioethanol Production by Enzymatic Hydrolysis from Different Lignocellulosic Sources. Molecules. 1 févr 2021;26(3):753.

3. Zheng Y, Zhao J, Xu F, Li Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. Progress in Energy and Combustion Science. juin 2014;42:35-53.

4. Amirta R, Tanabe T, Watanabe T, Honda Y, Kuwahara M, Watanabe T. Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, Ceriporiopsis subvermispora. Journal of Biotechnology. mai 2006;123(1):71-7.

5. Zhao C, Ma Z, Shao Q, Li B, Ye J, Peng H. Enzymatic Hydrolysis and Physiochemical Characterization of Corn Leaf after H-AFEX Pretreatment. Energy Fuels. 14 janv 2016;acs.energyfuels.5b02817.

6. Zheng J, Rehmann L. Extrusion Pretreatment of Lignocellulosic Biomass: A Review. IJMS. 20 oct 2014;15(10):18967-84.

7. Rosgaard L, Pedersen S, Meyer AS. Comparison of Different Pretreatment Strategies for Enzymatic Hydrolysis of Wheat and Barley Straw. Appl Biochem Biotechnol. 13 nov 2007;143(3):284-96.

8. Monlau F, Sambusiti C, Barakat A, Quéméneur M, Trably E, Steyer JP, et al. Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review. Biotechnology Advances. sept 2014;32(5):934-51.

9. Kim JS, Lee YY, Kim TH. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresource Technology. janv 2016;199:42-8.

10. Mathew AK, Parameshwaran B, Sukumaran RK, Pandey A. An evaluation of dilute acid and ammonia fiber explosion pretreatment for cellulosic ethanol production. Bioresource Technology. janv 2016;199:13-20.

11. Saha BC, Qureshi N, Kennedy GJ, Cotta MA. Biological pretreatment of corn stover with white-rot fungus for improved enzymatic hydrolysis. International Biodeterioration & Biodegradation. avr 2016;109:29-35.

12. Bensah EC, Kemausuor F, Miezah K, Kádár Z, Mensah M. African perspective on cellulosic ethanol production. Renewable and Sustainable Energy Reviews. sept 2015;49:1-11.

13. Duque A, Manzanares P, Ballesteros M. Extrusion as a pretreatment for lignocellulosic biomass: Fundamentals and applications. Renewable Energy. déc 2017;114:1427-41.

14. Karunanithy C, Muthukumarappan K. Influence of Extruder Temperature and Screw Speed on Pretreatment of Corn Stover while Varying Enzymes and Their Ratios. Appl Biochem Biotechnol. sept 2010;162(1):264-79.

15. Lee SH, Inoue S, Teramoto Y, Endo T. Enzymatic saccharification of woody biomass micro/nanofibrillated by continuous extrusion process II: Effect of hot-compressed water treatment. Bioresource Technology. déc 2010;101(24):9645-9.

16. da Silva AS, Teixeira RSS, Endo T, Bon EPS, Lee SH. Continuous pretreatment of sugarcane bagasse at high loading in an ionic liquid using a twin-screw extruder. Green Chem. 2013;15(7):1991.

17. Karunanithy C, Muthukumarappan K. Optimization of switchgrass and extruder parameters for enzymatic hydrolysis using response surface methodology. Industrial Crops and Products. janv 2011;33(1):188-99.

18. Cha YL, Yang J, Park Y, An GH, Ahn JW, Moon YH, et al. Continuous alkaline pretreatment of Miscanthus sacchariflorus using a bench-scale single screw reactor. Bioresource Technology. avr 2015;181:338-44.

19. Kuster Moro M, Sposina Sobral Teixeira R, Sant'Ana da Silva A, Duarte Fujimoto M, Albuquerque Melo P, Resende Secchi A, et al. Continuous pretreatment of sugarcane biomass using a twin-screw extruder. Industrial Crops and Products. mars 2017;97:509-17.

20. Gu Y, Zhang Y, Zhou X. Effect of Ca(OH)2 pretreatment on extruded rice straw anaerobic digestion. Bioresource Technology. nov 2015;196:116-22.

21. Kratky L, Jirout T. Biomass Size Reduction Machines for Enhancing Biogas Production. Chem Eng Technol. mars 2011;34(3):391-9.

22. Myat L, Ryu GH. Characteristics of destarched corn fiber extrudates for ethanol production. Journal of Cereal Science. sept 2014;60(2):289-96.

23. Han M, Kang KE, Kim Y, Choi GW. High efficiency bioethanol production from barley straw using a continuous pretreatment reactor. Process Biochemistry. mars 2013;48(3):488-95.

24. Kang KE, Han M, Moon SK, Kang HW, Kim Y, Cha YL, et al. Optimization of alkali-extrusion pretreatment with twin-screw for bioethanol production from Miscanthus. Fuel. juill 2013;109:520-6.

25. Luo L, van der Voet E, Huppes G. An energy analysis of ethanol from cellulosic feedstock–Corn stover. Renewable and Sustainable Energy Reviews. oct 2009;13(8):2003-11.

26. Heredia-Olea E, Pérez-Carrillo E, Montoya-Chiw M, Serna-Saldívar SO. Effects of Extrusion Pretreatment Parameters on Sweet Sorghum Bagasse Enzymatic Hydrolysis and Its Subsequent Conversion into Bioethanol. BioMed Research International. 2015;2015:1-10.

27. Amamou S, Sambusiti C, Monlau F, Dubreucq E, Barakat A. Mechano-Enzymatic Deconstruction with a New Enzymatic Cocktail to Enhance Enzymatic Hydrolysis and Bioethanol Fermentation of Two Macroalgae Species. Molecules. 17 janv 2018;23(1):174.

28. Barakat A, Chuetor S, Monlau F, Solhy A, Rouau X. Eco-friendly dry chemo-mechanical pretreatments of lignocellulosic biomass: Impact on energy and yield of the enzymatic hydrolysis. Applied Energy. 1 janv 2014;113:97-105.

29. Monlau F, Sambusiti C, Barakat A, Guo XM, Latrille E, Trably E, et al. Predictive Models of Biohydrogen and Biomethane Production Based on the Compositional and Structural Features of Lignocellulosic Materials. Environ Sci Technol. 6 nov 2012;46(21):12217-25.

30. Robertson JA, de Monredon FD, Dysseler P, Guillon F, Amado R, Thibault JF. Hydration Properties of Dietary Fibre and Resistant Starch: a European Collaborative Study. LWT - Food Science and Technology. mars 2000;33(2):72-9.

31. Huang YL, Ma YS. The effect of extrusion processing on the physiochemical properties of extruded orange pomace. Food Chemistry. févr 2016;192:363-9.

32. Aktas-Akyildiz E, Masatcioglu MT, Köksel H. Effect of extrusion treatment on enzymatic hydrolysis of wheat bran. Journal of Cereal Science. mai 2020;93:102941.

33. Zhang S, Xu Y, Hanna MA. Pretreatment of Corn Stover with Twin-Screw Extrusion Followed by Enzymatic Saccharification. Appl Biochem Biotechnol. janv 2012;166(2):458-69.

34. Haghighi-Manesh S, Azizi MH. Integrated extrusion-enzymatic treatment of corn bran for production of functional cake. Food Sci Nutr. oct 2018;6(7):1870-8.

35. Zhang Y, Li T, Shen Y, Wang L, Zhang H, Qian H, et al. Extrusion followed by ultrasound as a chemical-free pretreatment method to enhance enzymatic hydrolysis of rice hull for fermentable sugars production. Industrial Crops and Products. juill 2020;149:112356.

36. Wu W, Jiao A, Xu E, Chen Y, Jin Z. Effects of Extrusion Technology Combined with Enzymatic Hydrolysis on the Structural and Physicochemical Properties of Porous Corn Starch. Food Bioprocess Technol. mars 2020;13(3):442-51.

37. Zeng Z, Li Y, Yang R, Liu C, Hu X, Luo S, et al. The relationship between reducing sugars and phenolic retention of brown rice after enzymatic extrusion. Journal of Cereal Science. mars 2017;74:244-9.

38. Coimbra MC, Duque A, Saéz F, Manzanares P, Garcia-Cruz CH, Ballesteros M. Sugar production from wheat straw biomass by alkaline extrusion and enzymatic hydrolysis. Renewable Energy. févr 2016;86:1060-8.

39. Han SY, Park CW, Endo T, Febrianto F, Kim NH, Lee SH. Extrusion process to enhance the pretreatment effect of ionic liquid for improving enzymatic hydrolysis of lignocellulosic biomass. Wood Sci Technol. mai 2020;54(3):599-613.

40. Pérez-Rodríguez N, García-Bernet D, Domínguez JM. Extrusion and enzymatic hydrolysis as pretreatments on corn cob for biogas production. Renewable Energy. juill 2017;107:597-603.

41. Banvillet G, Gatt E, Belgacem N, Bras J. Cellulose fibers deconstruction by twin-screw extrusion with in situ enzymatic hydrolysis via bioextrusion. Bioresource Technology. mai 2021;327:124819.

42. Liu W, Chen W, Hou Q, Wang S, Liu F. Effects of combined pretreatment of dilute acid pre-extraction and chemical-assisted mechanical refining on enzymatic hydrolysis of lignocellulosic biomass. RSC Adv. 2018;8(19):10207-14.

43. Pereira MAF, Monteiro CRM, Pereira GN, Júnior SEB, Zanella E, Ávila PF, et al. Deconstruction of banana peel for carbohydrate fractionation. Bioprocess Biosyst Eng. févr 2021;44(2):297-306.

44. Merino ST, Cherry J. Progress and Challenges in Enzyme Development for Biomass Utilization. In: Olsson L, éditeur. Biofuels [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2007 [cité 23 mars 2021]. p. 95-120. (Advances in Biochemical Engineering/Biotechnology; vol. 108). Disponible sur: http://link.springer.com/10.1007/10_2007_066

45. Morgavi DP, Beauchemin KA, Nsereko VL, Rode LM, Iwaasa AD, Yang WZ, et al. Synergy Between Ruminal Fibrolytic Enzymes and Enzymes from Trichoderma Longibrachiatum. Journal of Dairy Science. juin 2000;83(6):1310-21.

46. Kunamneni A, Plou FJ, Alcalde M, Ballesteros A. Trichoderma Enzymes for Food Industries. In: Biotechnology and Biology of Trichoderma [Internet]. Elsevier; 2014 [cité 18 mai 2021]. p. 339-44. Disponible sur: https://linkinghub.elsevier.com/retrieve/pii/B9780444595768000242

47. Licari A, Monlau F, Solhy A, Buche P, Barakat A. Comparison of various milling modes combined to the enzymatic hydrolysis of lignocellulosic biomass for bioenergy production: Glucose yield and energy efficiency. Energy. mai 2016;102:335-42.

48. Yeh AI, Huang YC, Chen SH. Effect of particle size on the rate of enzymatic hydrolysis of cellulose. Carbohydrate Polymers. janv 2010;79(1):192-9.

49. Kapoor M, Semwal S, Satlewal A, Christopher J, Gupta RP, Kumar R, et al. The impact of particle size of cellulosic residue and solid loadings on enzymatic hydrolysis with a mass balance. Fuel. juin 2019;245:514-20.

50. Vandenbossche V, Brault J, Vilarem G, Hernández-Meléndez O, Vivaldo-Lima E, Hernández-Luna M, et al. A new lignocellulosic biomass deconstruction process combining thermo-mechano chemical action and bio-catalytic enzymatic hydrolysis in a twin-screw extruder. Industrial Crops and Products. avr 2014;55:258-66.

51. Xu H, Che X, Ding Y, Kong Y, Li B, Tian W. Effect of crystallinity on pretreatment and enzymatic hydrolysis of lignocellulosic biomass based on multivariate analysis. Bioresource Technology. mai 2019;279:271-80.

52. Yuan Y, Jiang B, Chen H, Wu W, Wu S, Jin Y, et al. Recent advances in understanding the effects of lignin structural characteristics on enzymatic hydrolysis. Biotechnol Biofuels. déc 2021;14(1):205.

53. Choi CH, Um BH, Oh KK. The influence of screw configuration on the pretreatment performance of a continuous twin screw-driven reactor (CTSR). Bioresource Technology. mars 2013;132:49-56.

54. Hu J, Arantes V, Saddler JN. The enhancement of enzymatic hydrolysis of lignocellulosic substrates by the addition of accessory enzymes such as xylanase: is it an additive or synergistic effect? Biotechnol Biofuels. 2011;4(1):36.

55. Akdogan H. Pressure, torque, and energy responses of a twin screw extruder at high moisture contents. Food Research International. juin 1996;29(5-6):423-9.

56. Chen WH, Xu YY, Hwang WS, Wang JB. Pretreatment of rice straw using an extrusion/extraction process at bench-scale for producing cellulosic ethanol. Bioresource Technology. nov 2011;102(22):10451-8.

57. Lu S, Wang Q, Liang Z, Wang W, Liang C, Wang Z, et al. Saccharification of sugarcane bagasse by magnetic carbon-based solid acid pretreatment and enzymatic hydrolysis. Industrial Crops and Products. févr 2021;160:113159.

58. Yang J, Gao C, Yang X, Su Y, Shi S, Han L. Effect of combined wet alkaline mechanical pretreatment on enzymatic hydrolysis of corn stover and its mechanism. Biotechnol Biofuels. déc 2022;15(1):31.

59. Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, et al. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover [Internet]. 2011 mars [cité 12 mai 2022] p. NREL/TP-5100-47764, 1013269. Report No.: NREL/TP-5100-47764, 1013269. Disponible sur: http://www.osti.gov/servlets/purl/1013269/

60. Kim S, Kim SD, Sohn SY. Evaluation of the wastewater generated during alkaline pretreatment of biomass for feasibility of recycling and reusing. Renewable Energy. août 2020;155:1156-64.

61. Hjorth M, Gränitz K, Adamsen APS, Møller HB. Extrusion as a pretreatment to increase biogas production. Bioresource Technology. avr 2011;102(8):4989-94.

Tables

Table 1 Twin-screw extrusion (TSE) experimental conditions.

Samples	Solid load (g TS /L)	Speed (rpm)
Untreated	-	-
CS (200/100)	200	100
CS (200/200)	200	200
CS (200/300)	200	300
CS (400/100)	400	100
CS (400/200)	400	200
CS (400/300)	400	300
CS (600/100)	600	100
CS (600/200)	600	200
CS (600/300)	600	300

Table 2 Main Physico-chemical properties of untreated and extruded corn stover. The coding of pretreated corn stover refers to CS (solid loading/screw speed). For each column, identical letters refer to insignificant differences (p> 0.05).

	Size D_{50} (μm)	**Surface area (m^2/g)**	**CrI (%)**	**Swelling (mL/g)**	**CS composition (g/100g TS)**			**Soluble sugars (mg/gTS)**
					Lignin	**Cellulose**	**Hemicelluloses**	
Untreated	Under detection limit			17.1±0.6 ^{a}	25.2±0.2^{a}	37.5±0.5 ^{a}	22.3±0.7 ^{a}	27.2±1.5 ^{a}
CS (200/100)	720.6	1.573	46	10.4±0.9 ^{b}	25.0±0.1 ^{a}	36.9±1.4 ^{a}	21.5±0.4 ^{a}	25.0±3.7 ^{a}
CS (200/200)	566.9	1.575	31	12.1±0.9 ^{c}	24.2±0.1 ^{a}	37.8±0.6 ^{a}	22.3±1.8 ^{a}	29.7±2.0 ^{a}
CS (200/300)	533.7	1.578	30	12.6±1.1 ^{c}	24.7±1.9 ^{a}	38.5±0.4 ^{ab}	21.6±0.1 ^{a}	29.1±2.3 ^{a}
CS (400/100)	464.9	1.697	35	9.4±1.2 ^{b}	24.8±0.9 ^{a}	37.1±2.9 ^{a}	21.4±0.3 ^{a}	19.3±0.7 ^{a}
CS (400/200)	708.4	1.698	38	9.7±1.1 ^{b}	24.9±0.1 ^{a}	37.4±4.4 ^{a}	21.9±0.4 ^{a}	25.0±1.4 ^{a}
CS (400/300)	940.6	1.606	35	9.7±0.4 ^{b}	23.1±0.4 ^{a}	38.3±0.7 ^{a}	21.9±1.1 ^{a}	29.2±1.7 ^{a}
CS (600/100)	675.3	1.661	31	8.7±1.2 ^{b}	24.3±0.8 ^{a}	39.7±0.1 ^{a}	22.1±0.7 ^{a}	25.5±2.4 ^{a}
CS (600/200)	559.4	1.681	34	8.2±0.4 ^{b}	23.8±0.8 ^{a}	39.9±0.8 ^{b}	22.7±0.0 ^{a}	25.8±0.9 ^{a}
CS (600/300)	423.4	1.671	35	8.2±0.4 ^{b}	24.5±1.3 ^{a}	36.4±4.9 ^{a}	21.5±0.6 ^{b}	22.1±2.0 ^{a}

Table 3 Effect of extrusion pretreatment on sugars solubilization in literature

Pretreatment	Biomass	Conditions	Soluble sugars	Ref
Twin-screw extrusion	Corn cob (50% of moisture)	200 kg/h Residence time:55 s At room temperature	+100%	(40)
Reactive extrusion		0.4% NaOH (w/v) 400 kg/h Residence time:35 s At room temperature	+70%	
Twin-screw extrusion	Wheat bran (3 mm)	105°C 12% of moisture 100 rpm	+65%	(32)
		105°C 12% of moisture 200 rpm	+130%	
Twin-screw extrusion	Corn bran	140°C 30% of moisture 300 rpm	+187%	(34)
		140°C 50% of moisture 300 rpm	+78%	
		120°C 30% of moisture 300 rpm	+153%	
		160°C 30% of moisture 300 rpm	+247%	
Reactive extrusion	Wheat straw	NaOH (10%, w/v) 70°C 12% of moisture 0.6 kg/h, 150 rpm, residence time= 2min	Glucan (13-fold) Xylan (11-fold)	(38)
Twin-screw extrusion	Corn starch	Around 70°C 6 kg/h (33% of moisture) 120 rpm	Glucose (+38%)	(36)
Reactive extrusion	Willow (*Salix gracilistyla* Miq)	The ratio of [EMIM]Ac (50%) solid content 50%, extrusion temperature 140 °C screw rotating speed (5 rpm).	Glucan (40.1%) Xylan (11.6%)	(39)
Bio-extrusion	Brown rice	60°C moisture 30%, 100 rpm α-amylase (0.5 %, w/w)	300 mg/g of biomass	(37)
Extrusion		60°C moisture 30%, 100 rpm	9 mg/g of biomass	
Combined extrusion and ultrasonic	Rice hull	143°C moisture 29%, 350 rpm	346 mg/g of biomass	(35)
Twin-screw extrusion	Straw	33°C 55 kW	No effect on cellulose, hemicelluloses, and lignin.	(61)
Twin-screw extrusion	Corn stover	37°C 400 gTS/kg and 200 rpm	No effect on solubilization	This study

Figures

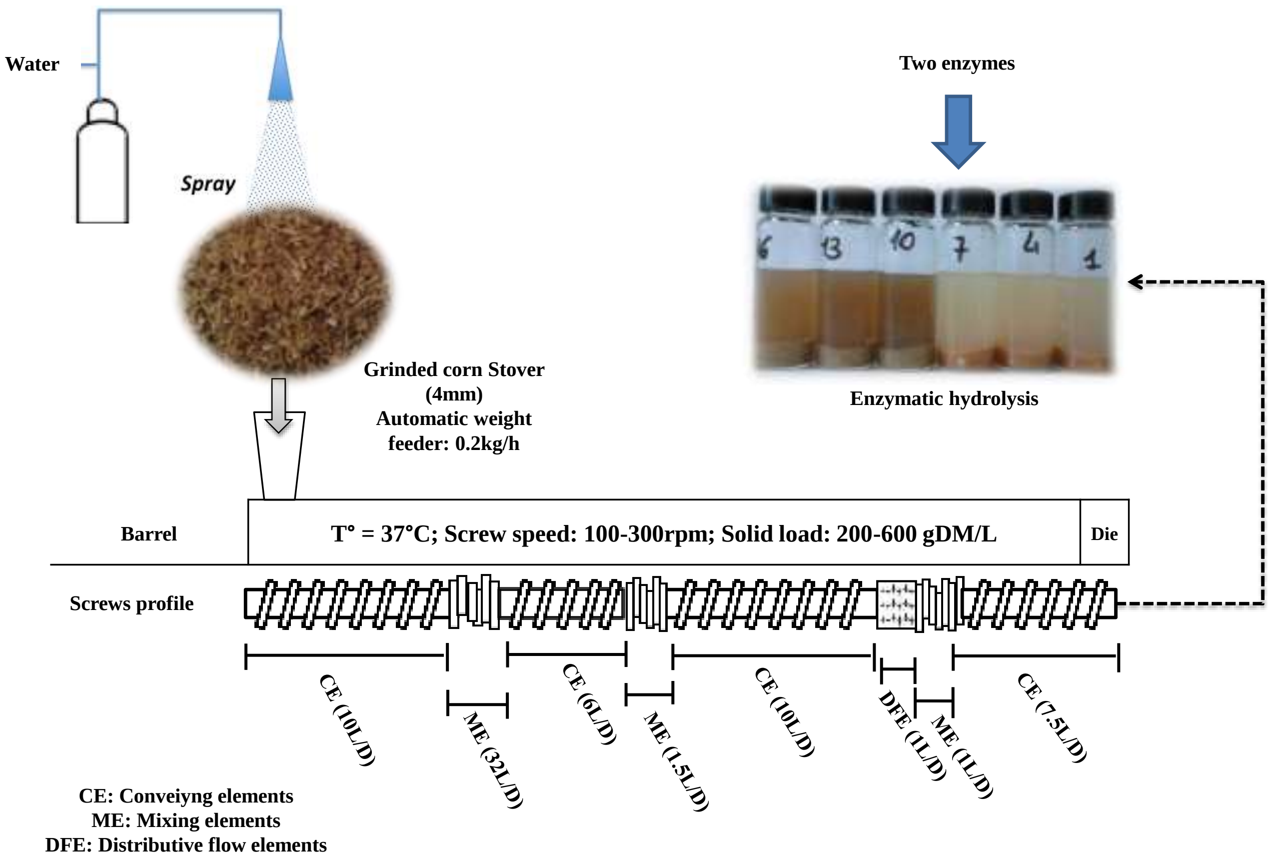
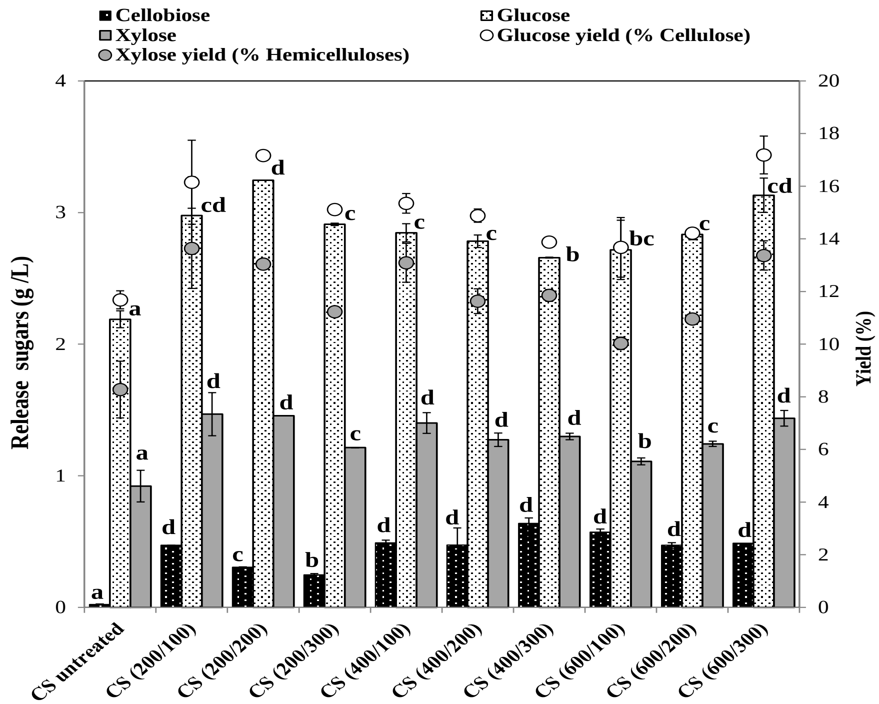


Fig.1 Scheme of the experimental procedure used in this study

a)



b)

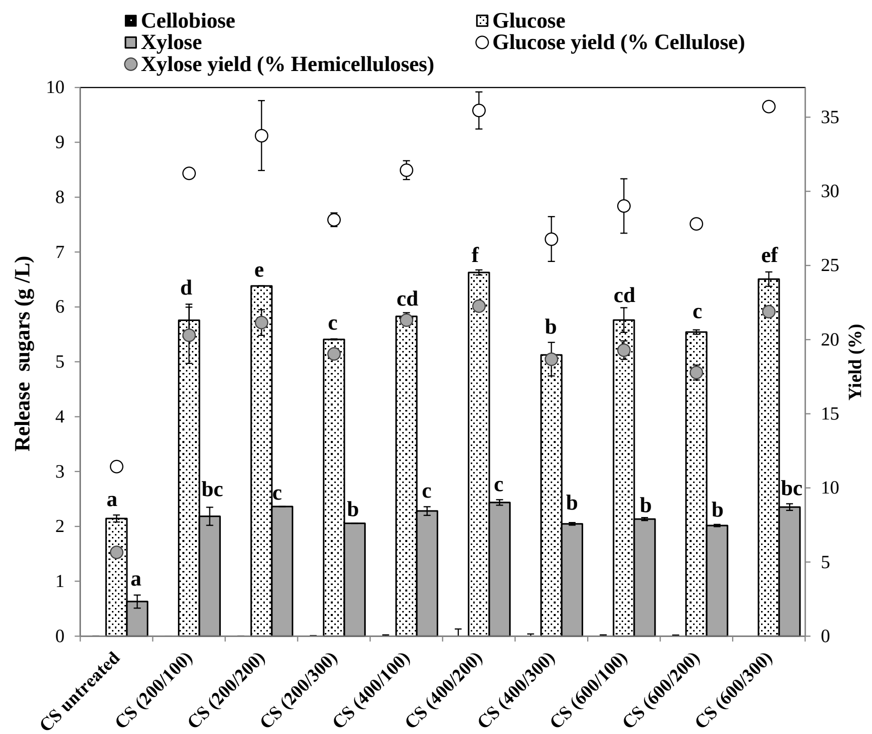


Fig.2 Released sugars and their yields after different extrusion conditions: a) using cellulase from *T. reesei*; b) using cellulase from *T. longibrachiatum*. The coding of pretreated corn stover refers to CS (solid loading/screw speed).

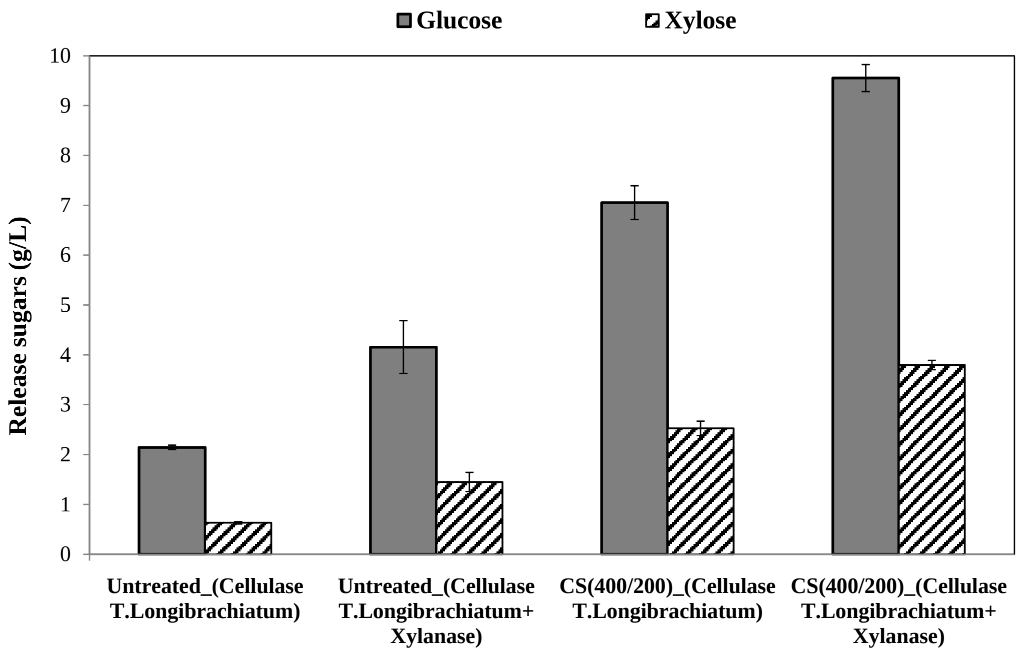


Fig.3 Released sugars from untreated and pretreated corn stover (under 400 gTS/kg and 200 rpm) with xylanase addition

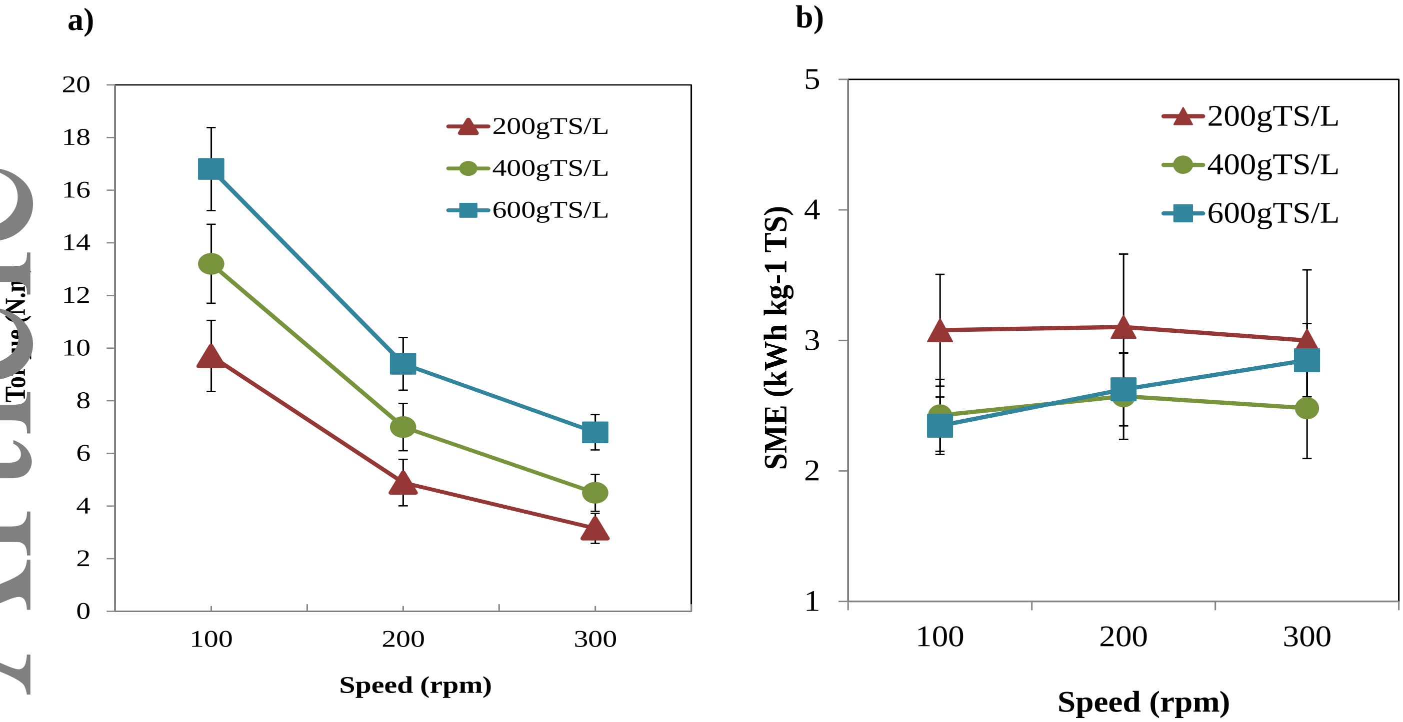


Fig.4 Impact of extrusion conditions on: a) Torque and b) specific mechanical energy consumption

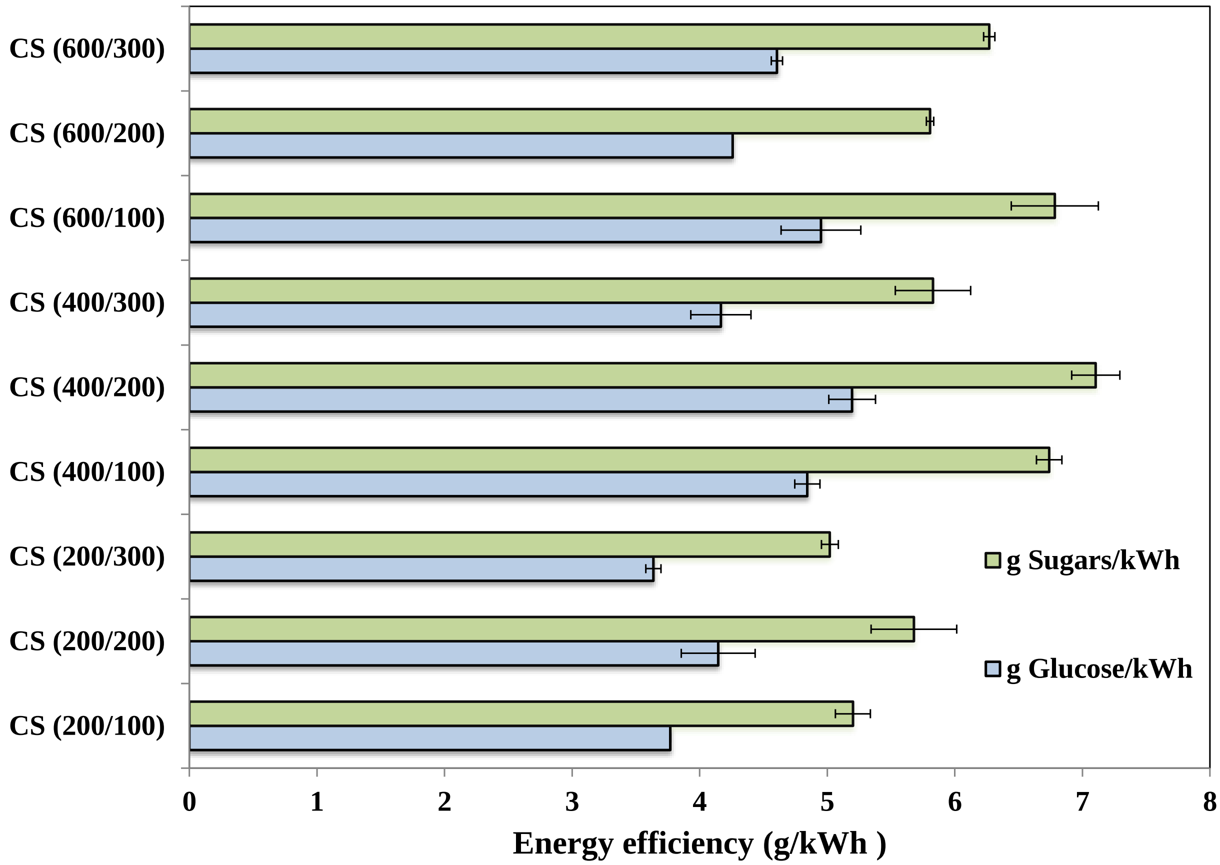


Fig.5 Energy efficiency (g. kWh-1) of sugars production after hydrolysis enzymatic of CS biomass of different TSE experimental conditions. The coding of pretreated corn stover refers to CS (solid loading/screw speed).